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Effects of steaming on health-valuable nutrients from fortified farmed fish: Gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) as case studies

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ABSTRACT

Fish fortification with iodine-rich macroalgae (*Laminaria digitata*) and Selenium-rich yeast is expected to promote nutritional added value of this crucial food item, contributing to a healthy and balanced diet for consumers. However, it is not known if steaming can affect these nutrient levels in fortified fish. The present study evaluates the effect of steaming on nutrients contents in fortified farmed gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*). Fortified seabream presented enhanced I, Se and Fe contents, whereas fortified carp presented enhanced I, Se and Zn contents. Steaming resulted in increased I and Se contents in fortified seabream, and increased Fe and Zn levels in fortified carp, with higher elements true retention values (TRVs >90%). The consumption of 150 g of steamed fortified seabream contributes to a significant daily intake (DI) of I (up to 12%) and Se (up to >100%). On the other hand, steamed fortified carp contributes to 19–23% of I DI and 30%–71% of Se DI. These results demonstrate that steaming is a healthy cooking method, maintaining the enhanced nutritional quality of fortified fish. Moreover, the present fortification strategy is a promising solution to develop high-quality farmed fish products to overcome nutritional deficiencies.

1. Introduction

The 21st century global challenges include those related with environmental changes and worldwide population nutritional deficiencies (United Nations, 2020). Several scientific evidences demonstrate that seafood consumption have been associated with beneficial effects for human health, when consumed at least twice a week (EFSA, 2015a; Luten et al., 2008). Fish contains many nutrients required to address micronutrient deficiencies (i.e. iodine, iron and selenium) that affects 30% of the world's population (FAO, 2008). In addition, several evidences stress the beneficial health effects of fish consumption in mental health and in the prevention of cardiovascular diseases (Luten et al., 2008; Pinkaew and Karrila, 2015). Currently, there is a growing trend to develop tailor-made fish products by including natural ingredients with health-promoting nutrients in order to meet consumers' nutritional

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Abbreviations

AI	Adequate Intake
AR	Adequate Requirement
As	Arsenic
BF1	Fortified B1
BF2	Fortified B2
Br	Bromide
Ca	Calcium
Cl	Chlorine
CTR	Control
Cu	Copper
CY	Cooking Yield
DHA	Docosahexaenoic acid (22:6*n*-3)
DORM-4	Fish protein certified reference material
DRVs	Dietary reference values
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid (20:5*n*-3)
ERM®-BB422	Fish muscle certified reference material
Fe	Iron
I	Iodine
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
IRMM	Institute for Reference Materials and Measurements
K	Potassium
LOD	Limit of Detection
LOQ	Limit of Quantification
NC	Nutritional Contribution
PCA	Principal Component Analysis
PTWI	Provisional Tolerable Weekly Intake
Se	Selenium
SRM 1571	Oyster tissue certified reference material
TMAH	Tetramethylammonium hydroxide
TR	True Retention
TRV	True Retention Values
UL	Upper Intake Level
USDA	United States Department of Agriculture
XRF	X-ray Fluorescence
μ-EDXRF	Micro-Energy Dispersive X-ray Fluorescence Spectrometry

requirements and the growing health consciousness for sustainable, natural, safe and high-quality food (FAO, 2018). Several studies demonstrate that the natural enhancement of aquaculture feeds with health-promoting nutrients is an important strategy to produce sustainable, healthy/nutritious fortified farmed fish products (Barbosa et al., 2020; Cotter et al., 2009; Ramos et al., 2008; Ribeiro et al., 2015, 2017; Saltzman et al., 2013; Valente et al., 2015). Within the context of functional food, fortified fish products are a potential strategy to improve consumers diets, providing beneficial health effects beyond the provision of essential nutrients (e.g., vitamins and minerals), when consumed as part of a diversified diet approach (Hasler, 2002; Luten et al., 2008). Nevertheless, the success of fish fortification as functional food depends on the combination of its efficacy (enhancement of active components linked to increased health benefits and disease risk reduction) and consumes' acceptance (Hasler, 2002; Ribeiro et al., 2019). Moreover, consumer's demand for healthier, natural and cost-effective fortified farmed fish products, foster the aquaculture sector to design and produce novel fish products using more sustainable and natural ingredients in feeds formulation (Ribeiro et al., 2019). The use of different ingredients from algae and plant or non-animal sources in fish feed formulation, especially I-rich seaweed, EPA and DHA-rich microalgae and Se-rich yeast, plays an important role in the aquaculture sector, promoting the development of eco-innovative fortified fish products and the reduction of production costs and wastes (FAO, 2018; Sidari and Tofalo, 2019). A previous study demonstrated the efficacy of fish fortification with health-valuable nutrients through the incorporation of I-rich seaweed (*Laminaria digitata*) and Se-rich yeast in gilthead seabream and common carp feeds, resulting in enhanced I, Se and iron (Fe) contents in fish muscle, without compromising consumer safety (Barbosa et al., 2020). Indeed, the replacement of fishmeal and fish oil by microalgae blends, I-rich macroalgae and Se-rich yeast result in less exposure to toxic elements, mainly Hg, Cd and Pb (Barbosa et al., 2020).

Gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) are two of the most intensively farmed species in Europe, being mostly produced and consumed in Mediterranean countries and in central Europe, respectively (European Union, 2019). As a matter of fact, gilthead seabream and common carp represents, respectively 10% and 5% of European aquaculture production, sharing 7% (gilthead seabream) and 6% (common carp) of European total apparent consumption (EUMOFA, 2019). Despite the increase trend in global fish consumption and the beneficial effects associated with seafood diets, I deficiency is a major concern of European authorities with critical consequences in neurological development, especially in children (FAO, 2018; WHO, 2013). Moreover, Se deficiency has been implicated in cardiovascular diseases, infertility and hypothyroidism (Martins et al., 2011), while Fe deficiency is one of the world's most common disorders that lead to anaemia (Kongkachuichai et al., 2002). Since I and Se are not naturally found in the human body, the main source of these minerals for humans is the diet, particularly seafood (Bevis, 2015).

In general, most seafood is only consumed after cooking and therefore it is important to take into consideration the diversity and effect of culinary procedures when estimating nutrients daily intakes. Several culinary methods, such as boiling, grilling, frying, steaming and roasting, are usually used to cook fish before consumption, and vary according to the region, local traditions and cultural heritages (Sobral et al., 2018). Although cooking procedures improves fish digestibility and safety in terms of pathogenic microorganisms (Oliveira et al., 2019; Sobral et al., 2018), it can also lead to potential changes in the nutritional value (Alves et al., 2018; Karimian-Khosroshahi et al., 2016; Oliveira et al., 2019; Tontisirin et al., 2002). Indeed, the content of nutrients in cooked fish may increase or decrease compared to the raw counterpart, depending on the culinary procedures used (Badiani et al., 2013; Karimian-Khosroshahi et al., 2016). Overall, thermal processing is associated to water-soluble nutrients (i.e. vitamins C and B) leaching (Karimian-Khosroshahi et al., 2016). Regarding minerals, both increases or decreases in its content (i.e. Ca, Cu, Fe, I and Se) have been reported in fish, though varying with fish species and cooking methods (Alves et al., 2018; Sobral et al., 2018). For example, steaming results in increased Zn (hake, mackerel, plaice and seabream), Se (mussels and octopus), Na, K, Fe and Cu (seabream) contents (Alves et al., 2018; Mnari et al., 2012). On the other hand, boiling and microwave cooking results in decreased K and increased Zn contents in rainbow trout, while grilling results in increased Cu content in seabream (Gokoglu et al., 2004; Mnari et al., 2012). Still, steaming has been pointed out as the healthier option and generally inducing less changes in the product nutritional content compared to other culinary procedures such as frying or grilling (Alves et al., 2018; Maulvault et al, 2012, 2013).

The effects of culinary treatments on enhanced health-valuable nutrients in fortified fish products have not been previously studied. Moreover, most available studies assessing the effects of cooking methods on nutrient contents in seafood did not consider the use of the true retention values (TRVs) approach, which allows to provide more accurate knowledge on nutrients content after culinary procedures (Bognár and Piekarski, 2000). Hence, the present work aims to: (1) evaluate the effects of steaming on essential nutrients contents (i.e. I, Se, Cu, Zn, Fe, Ca, K) in gilthead seabream and common carp fish muscle

(fillets) fortified with I-rich seaweed (*L. digitata*) and Se-rich yeast as feed ingredients; and (2) provide the most accurate data on nutrients contribution to the dietary reference values (DRVs) by using true retention (TR) calculations.

2. Material and methods

2.1. Experimental diets

For each species, three experimental diets were formulated, a control diet (CTR), considering the nutritional requirements of adult gilthead seabream and common carp, and two enriched diets supplemented with different blends of I-rich macroalgae and Se-rich yeast (BF1 and BF2, respectively). Based on the control formulation, gilthead seabream enriched diets were formulated targeting increased I levels, supplied from *L. digitata* (0.40% in BF1 and 0.80% in BF2) and increased Se levels, supplied through Se-rich yeast (0.02% in BF1 and 0.04% in BF2). Additionally, enriched seabream diets were formulated with a 5% replacement of fishmeal by a blend of microalgae (*Tetraselmis* sp., *Chlorella* sp., *Schizochytrium* sp.) and with the reduction of vegetable oils levels (1.05% in BF1 and 2.15% in BF2). The enriched BF1 diet also contained less fish oil (1.09%; Table 1). Concerning common carp, the enriched diets were formulated based on control diet (CTR), targeting increased I levels, supplied from *L. digitata* (0.54%) and increased Se levels, supplied from Se-rich yeast (0.01%). Enriched carp diets were formulated with a 2.5% replacement of fishmeal by a blend of microalgae (*Spirulina* sp. and *Chlorella* sp.) and the enriched BF1 diet was supplemented with DHA-rich microalgae (1.56% *Schizochytrium* sp.), whereas the enriched BF2 diet was supplemented with salmon oil (2.10%) from salmon industry by-products. In addition, the enriched BF1 diet contained higher levels of rapeseed oil (5.1%) and lower levels of soybean oil (0%), whereas the enriched BF2 diet contained lower levels of rapeseed and soybean oils (2%; Table 1). Experimental extruded diets were manufactured by SPAROS, Ltda (Olhão, Portugal) and the enriched diets formulations took into consideration the current maximum authorized contents of total I (20 mg kg^{-1}) and Se (0.5 mg kg^{-1}) in fish feeds (EFSA, 2014a,b).

2.2. Growth trial and sampling

The trial with gilthead seabream was conducted at the Aquaculture Research Station (EPPO-IPMA, Olhão, Portugal) of IPMA, whereas the common carp trial was conducted at the Fisheries Research Station (FRS-ZUT Nowe Czarnowo, Poland). Both trials were performed in compliance with the European guidelines on protection of animals used for scientific purposes (European Commission, 2007).

The experimental design is schematized in Fig. 1. Nine homogenous groups of 50 gilthead seabream each, with a mean initial body weight of 374 ± 9 g were distributed in 1500 L circular fiberglass tanks, supplied with flow-through seawater circulation (salinity: 35‰; temperature: 24–25 °C; dissolved oxygen 5.6 ± 0.9 mg L^{-1}) and subjected to natural photoperiod summer conditions (14 h light/10 h dark). Each experimental treatment was tested in triplicate tanks (n = 150 fish per treatment) over 72 days. Common carp specimens, with a mean initial body weight of 296 ± 10 g were distributed in a floating set of nine cages with 3000 L each (n = 100 fish per cage), placed in the cooling water discharge channel of the Dolna Odra power plant. Each experimental treatment was tested in triplicate tanks (n = 300 fish per treatment) over 98 days. For each species, fish were hand-fed to apparent satiety in three to four daily meals with 1.3–2.0% of the biomass. during the experimental period, mimicking the final stage of the production (i.e. just before reaching market size). No mortality was observed during either trial. Final samplings were done 24 h following the last meal and 15 fish per treatment (5 per replicate tank or cage) were sacrificed by immersion in chilled seawater (gilthead seabream) or freshwater (common carp) following the commercial procedures employed in fish farms. Both

Table 1
Ingredients and proximate composition (%) of the experimental diets (CTR - control, BF1 – fortified diet B1, BF2 - fortified diet B2) for gilthead seabream (*S. aurata*) and common carp (*C. carpio*).

	Gilthead seabream			Common carp		
Ingredients (%)	CTR	BF1	BF2	CTR	BF1	BF2
Fishmeal 70[1]	15.00	10.00	10.00	–	–	–
Fishmeal 60[2]	–	–	–	5.00	2.50	2.50
Fish protein concentrate[3]	2.50	2.50	2.50	–	–	–
Porcine blood meal[4]	2.50	2.50	2.50	2.00	2.00	2.00
Algae meal (*Tetraselmis* sp.)[5]	–	0.50	0.50	–	–	–
Algae meal (*Spirulina* sp.)[6]	–	–	–	–	1.00	1.00
Algae meal (*Chlorella* sp.)[7]	–	5.00	5.00	–	1.00	1.00
Algae meal (*Schizochytrium* sp.)[8]	–	3.20	3.20	–	1.56	–
Soy protein concentrate[9]	17.00	17.00	17.00	2.50	2.50	2.50
Corn gluten meal[10]	8.00	8.00	8.00	4.00	4.00	4.00
Soybean meal 48[11]	8.00	8.00	8.00	–	–	–
Soybean meal 44[12]	–	–	–	25.00	25.00	25.00
Rapeseed meal[13]	–	–	–	7.00	7.00	7.00
Sunflower meal[14]	–	–	–	12.50	12.50	12.50
Corn meal[15]	–	–	–	2.50	2.50	2.50
Wheat meal[16]	16.60	14.40	14.00	22.50	21.80	22.40
Wheat glúten[17]	12.00	12.00	12.00	–	–	–
Wheat bran[18]	–	–	–	5.00	5.00	5.00
Fish oil[19]	5.45	4.36	5.45	–	–	–
Salmon oil[20]	–	–	–	–	–	2.10
Soybean oil[21]	2.81	2.49	2.16	3.00	–	2.00
Rapeseed oil[21]	5.61	4.98	4.32	3.00	5.10	2.00
Linseed oil[21]	0.94	0.83	0.72	–	–	–
Vitamins and minerals premix[22]	1.10	1.10	1.10	1.00	1.00	1.00
Betaine HCl[23]	–	–	–	0.10	0.10	0.10
Binder[24]	1.00	1.00	1.00	1.00	1.00	1.00
Macroalgae meal (*Laminaria digitata*)[25]	–	0.40	0.80	–	0.54	0.54
Antioxidant[26]	0.20	0.20	0.20	0.20	0.20	0.20
Sodium propionate[27]	0.10	0.10	0.10	0.10	0.10	0.10
Monoammonium phosphate[28]	0.50	0.50	0.50	–	–	–
Sodium phosphate[29]	–	–	–	2.10	2.10	2.10
Selenised yeast[30]	–	0.02	0.04	–	0.01	0.01
L-Taurine[31]	0.40	0.50	0.50	–	–	–
L-Tryptophan[32]	0.10	0.10	0.10	0.20	0.20	0.20
DL-Methionine[33]	0.20	0.30	0.30	0.60	0.60	0.60
L-Lysine[34]	–	–	–	0.70	0.70	0.70
Dry matter (DM), %	7.90 ± 0.00	8.10 ± 0.00	8.10 ± 0.01	5.30 ± 0.01	6.40 ± 0.02	8.30 ± 0.02
Crude protein, % DM	46.00 ± 0.10	45.70 ± 0.20	45.50 ± 0.10	30.20 ± 0.20	30.40 ± 0.10	30.30 ± 0.10
Crude fat, % DM	17.20 ± 0.10	17.30 ± 0.10	17.30 ± 0.10	8.10 ± 0.10	8.00 ± 0.10	8.10 ± 0.20
Ash, % DM	5.30 ± 0.00	5.30 ± 0.01	5.30 ± 0.01	4.40 ± 0.10	7.20 ± 0.20	7.20 ± 0.10
Iodine, mg kg^{-1} DM	1.24 ± 0.02	7.38 ± 0.66	13.3 ± 0.2	2.22 ± 0.03	16.30 ± 0.30	15.60 ± 0.30
Selenium, mg kg^{-1} DM	0.70 ± 0.00	1.05 ± 0.01	1.28 ± 0.02	0.40 ± 0.01	1.47 ± 0.05	1.41 ± 0.05

[1]CONRESA 70: 47.4% crude protein (CP), 817.5% crude fat (CF), Conserveros Reunidos S.A., Spain; [2]CONRESA 60: 61.2% crude protein (CP), 8.4% crude fat (CF), Conserveros Reunidos S.A., Spain; [4]Porcine blood meal: 89% CP, 1% CF, SONAC BV, The Netherlands; [5]Tetraselmis meal: 72% CP, 1% CF, Willows Ingredients Ltd, Ireland; [6]Spirulina meal: 72% CP, 1% CF, Willows Ingredients Ltd, Ireland; [7]Chlorella meal: 62% CP, 9% CF, ALLMICROALGAE, Portugal; [8]ALL-G RICH (Schizochytrium), Alltech Portugal; [9]Soycomil P: 63% CP, 0.8% CF, ADM,

The Netherlands; [10]Corn gluten meal: 61% CP, 6% CF, COPAM, Portugal; [11]Solvent extracted soybean meal: 43.8% CP, 3.3% CF, CARGILL, Spain; [12]Solvent extracted soybean meal: 43.8% CP, 3.3% CF, CARGILL, Spain; [13]Defatted rapeseed meal: 32.7% CP, 4.1% CF, Ribeiro & Sousa Lda, Portugal; [14]Defatted sunflower meal: 29.1% CP, 1.8% CF, Ribeiro & Sousa Lda, Portugal; [15]Corn meal: 8% CP, 3.7% CF, Ribeiro & Sousa Lda, Portugal; [16]Wheat meal: 10.2% CP, 1.2% CF, Casa Lanchinha, Portugal; [17]Wheat glúten; [18]Wheat bran: 14.9% CP, 4.0% CF, Cerealis Moagens S.A., Portugal; [19]Fish oil; [20]Sopropêche; [21] H Lamotte Oils GmbH, Germany; [22]INVIVONSA Portugal SA, Portugal: Vitamins (IU or mg/kg diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20000 IU; DL-cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 500 mg; inositol, 500 mg; biotin, 3 mg; calcium panthotenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg/kg diet): copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate,7.5 mg; sodium chloride, 400 mg; excipient wheat middling's; [23]ORFFA, The Netherlands; [24]CELATOM FP1SL (diatomite), Angelo Coimbra S. A., Portugal; [25]Dry Laminaria digitata: 5.4% CP, 0.5% CF, 3700 mg iodine/kg, Agrimer, France; [26]VERDILOX, Kemin Europe NV, Belgium; [27]PREMIX LDA., Portugal; [29]Vadequimica, Spain; [30]ALKOSEL R397: 2200 mg selenium/kg, Lallemand, France; [31]L-Taurine; [32]TrypAMINO 98%, Evonik Nutrition & Care GmbH, Germany; [33]DL-METHIONINE FOR AQUACULTURE 99%, EVONIK Nutrition & Care GmbH, Germany; [34]L-Lysine HCl 99%: Ajinomoto Eurolysine SAS, France.

gilthead seabream and common carp skinless fish muscle were collected at the start and at the end of the trial (n = 3 pools of 5 fish each). All fish were measured, weighted (morphometric data in Supplementary Table 1) and at the end of the trial one fish fillet collected was used for culinary steam-cooking procedure assessment (steaming) and the other fillet for raw assessment. All fish samples were homogenized with a grinder (Retasch Grindomix GM200, Germany) using polypropylene cups and stainless-steel knives at 10,000 *g* until complete visual disruption of the tissue and stored at −80 °C until further analysis.

2.3. Culinary steam-cooking procedure

For each treatment and species, fish muscle was individually wrapped up in aluminium foil and steamed in an oven (Combi-Master CM 6, Rational GroßkÜcken Technik GmbH, Germany) at 105 °C during 15 min. After steaming, fish muscle samples were cooled at room temperature. The final weight was registered to obtain the relevant cooking yield (CY = 100 × steamed weight/raw weight), as the percentage ratio between cooked and raw fish muscle weight (Supplementary Table 1).

2.4. Analytical methods

2.4.1. Elemental composition

Iodine (I), selenium (Se) and arsenic (As) were determined in fish muscle samples by inductively coupled plasma mass spectrometer (ICP-MS; Thermo X series II, Thermo Fisher Scientific, Waltham, USA) according to Barbosa et al. (2020). Iodine (I) content was quantified according to the EN 15111:2007 (European Standard, 2007) and Se and As according to the EN 15763:2009 (European Standard, 2009). Briefly, the alkaline digestion (for I) was performed by a 48-well graphite heating block (DigiPREP, SCP Science, Courtaboeuf, France) with tetramethylammonium hydroxide (TMAH; Fluka, St. Gallen, Switzerland) solution 25% (v/v), whereas the acid digestion (for Se and As) was performed overnight with 60% (v/v) ultrapure nitric acid solution, followed by a 48-well graphite heating block (DigiPREP, SCP Science, Courtaboeuf, France) with hydrogen peroxide solution 30% (v/v, Merck). ICP-MS operating conditions were optimized daily, and the quantification was done by linear calibration using standard solutions of I, Se and As prepared from single elements high purity ICP stock standards (Inorganic Ventures and SCP Science, respectively), ranging between 1 and 50 μg g^{-1} for I, 0.5 and 5 μg g^{-1} for Se and 0.25 and 2.5 μg g^{-1} for As (Coelho et al., 2017; Delgado et al., 2019).

Chlorine (Cl), potassium (K), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn) and bromide (Br) were determined by micro-energy dispersive X-ray fluorescence spectrometry (μ-EDXRF) according to Reboredo et al. (2020). Briefly, feed and freeze-dried fish muscle samples were dried and ground for 2 min under 10 tons pressure in order to make a cylindrical pellet with a diameter of 20 mm and a thickness of 1 mm. The energy μ-EDXRF spectra were acquired by a polarized geometry, secondary target and high energy XRF spectrometer. The characteristic radiations emitted by each element in the sample were detected by a Si (Li) detector with 30 mm^2 of sensitive area, 142 eV resolution at 5.9 keV cooled by liquid nitrogen. The acquisition time of each spectrum was adjusted for each secondary target and the operating conditions of the X-ray tube were 50 kV, 300 μA. The spectra were evaluated using the fundamental parameters method.

2.4.2. Quality assurance

All reagents used in the analyses were of high analytical grade and water was ultra-purified (<18 MΩ cm) using a Milli-Q-Integral system (Merck, Germany). Analytical quality was assessed through reference materials including Oyster tissue (SRM 1571) from the National Institute of Standards and Technology (Gaithersburg, EUA) and fish muscle (ERM®-BB422) from the European Commission – Joint Research Centre Institute for Reference Materials and Measurements (IRMM) (Geel, Belgium). The obtained values were in agreement with certified values. Detailed information about quality assurance, including the limit of quantification (LOQ) and detection (LOD), are shown in Table 2.

2.5. True retention (TR)

The TR (%) for each element was calculated using the following formula (USDA, 2007): TR = (mean content of the element in cooked food)/(mean content of the element in raw food) × CY.

2.6. Nutritional contribution (NC)

The NC of steamed fish muscle was calculated considering the consumption of 150 g of fish and the dietary reference values (DRVs) recommended by the European Food Safety Authority (EFSA), according to the following formula:

$$NC\ (\%) = 100 \times (C \times M) / DRV$$

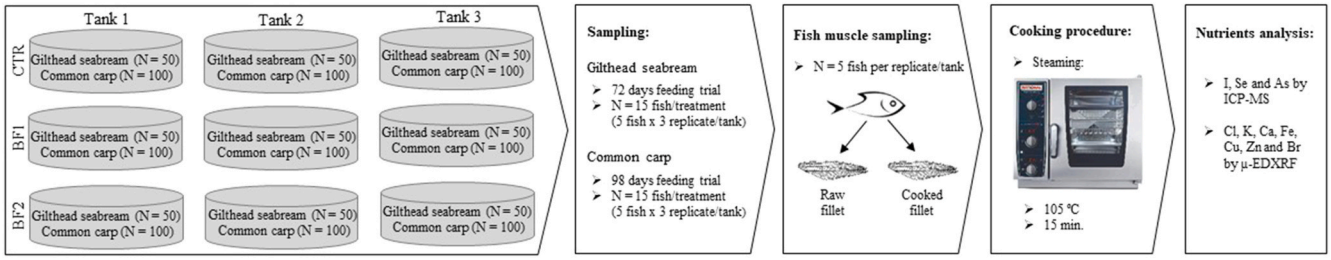

Fig. 1. Experimental design.

Table 2
Average certificate and measured concentrations (μg g^{-1} dry matter) and the associated relative standard deviation (RSD) in certified reference materials (CRM). Limit of detection (LOD) and limit of quantification (LOQ) for each element and analytical method.

Elements	Analytical method	CRM			LOD	LOQ
		Type	Certificate value (μg g^{-1})	Measured value (μg g^{-1})	(μg g^{-1})	(μg g^{-1})
As	ICP-MS	ERM®-BB422	12.7 ± 0.7	12.0 ± 0.2	0.003	0.013
I[a]	ICP-MS	ERM®-BB422	1.40 ± 0.40	1.23 ± 0.02	0.010 (0.068)	0.036 (0.25)
Se	ICP-MS	ERM®-BB422	1.33 ± 0.13	1.21 ± 0.02	0.007	0.025
Cl	μ-EDXRF	SRM 1571	700	600 ± 100	100	–
K	μ-EDXRF	SRM 1571	14700 ± 300	13500 ± 1300	20	–
Ca	μ-EDXRF	SRM 1571	20900 ± 300	19500 ± 2000	30	–
Fe	μ-EDXRF	DORM-4	142 ± 10	150 ± 15	2	–
		SRM 1571	12 ± 1	13 ± 1		
Cu	μ-EDXRF	DORM-4	2.3 ± 0.2	2.4 ± 0.8	1	–
		SRM 1571	12 ± 1	13 ± 1		
Zn	μ-EDXRF	DORM-4	27 ± 2	28 ± 3	1	–
		SRM 1571	25 ± 3	24 ± 2		
Br	μ-EDXRF	SRM 1571	10	11 ± 1	1	–

ICP-MS (Inductively coupled plasma mass spectrometer); μ-EDXRF (micro energy dispersive X-ray fluorescence spectrometry); ERM®-BB422 (Fish muscle CRM, Joint Research Centre (JRC), Brussels); SRM 1571 (Orchard leaf National Institute of Standards and Technology, EUA); DORM-4 (Fish Protein CRM, National Research Council of Canada, Canada).

[a] Iodine values for fish matrix and in parentheses for feed matrix.

where C = concentration of the element in mg kg^{-1}; M = typical meal portion in kg (0.150 kg for adults and pregnant women and 0.075 kg for children); DRV = adequate intake (AI; mg day^{-1}) for I, Se, Fe, Cu, Cl or K (EFSA, 2014a,b, 2015b,c, 2016, 2019) and adequate requirement (AR; mg day^{-1}) for Ca or Zn (EFSA, 2014c; 2015d). Since the reference value for total As (PTWI of 15 μg kg^{-1} body weight) is no longer appropriate (EFSA, 2009), and the most toxic and regulated form of As (i.e. inorganic As) was not analysed, this element was not included in these approach. Moreover, Br was not considered either, as no reference value is available.

2.7. Statistical analysis

Data were analysed for distribution and variance homoscedasticity using Kolmogorov–Smirnov and Levene's tests, respectively. The *t*-test student for dependent samples was performed to test significant differences between elements content in raw and steamed fish, for each treatment (CTR, BF1 and BF2). Whenever data (or transformed data) did not meet the normality and variance homoscedasticity assumptions, non−parametric Mann–Whitney *U* test was used. Furthermore, differences in fish muscle elements content among treatments (CTR, BF1 and BF2) were analysed by One-way ANOVA, followed by Tukey's post-hoc test for pair wise multiple comparisons. When ANOVA assumptions were not met, the Kruskal–Wallis test was performed followed by non-parametric multiple comparison test. Significance level was assigned at 0.05. Samples were also discriminated by multivariate parametric methods where the principal component analysis (PCA) was carried out to compute the linear combinations of the elements retained in each treatment. All analyses were carried out using STATISTICA™ (Version 7.0, StatSoft Inc., Tulsa, Oklahoma, USA).

3. Results

3.1. Essential elements composition in raw and steamed fortified farmed fish

Significantly higher CY were observed in fortified gilthead seabream (84% for BF2 and 87% for BF1) compared to fortified common carp (80% for BF2 and 81% for BF1) (Supplementary Table 1).

Fortified gilthead seabream (BF1 and BF2) presented significantly higher contents of I and Se, compared to the CTR (Table 3). Additionally, higher contents of Fe (BF1 and BF2) and Zn (BF1) were found in fortified fillets, compared to the CTR. On the other hand, fortified BF2 fillets presented significantly lower contents of Cu and Br (<LOD) compared to CTR fillets, while fortified BF1 fillets presented significantly higher contents of Cu and Br compared to CTR fillets. Steaming significantly increased I content in gilthead seabream fillets in all treatments (CTR, BF1 and BF2), as well as Se content in fortified BF2 fillets, resulting in TRs above 100% and 93%, respectively. Contrarily, Fe content significantly decreased in fortified BF2 fillets after steaming (69% TR), while Cu and Br contents significantly decreased in fortified BF1 and CTR reaching levels below LOD). Concerning macro elements, fortified gilthead seabream (BF2) presented significantly higher Cl contents compared to the CTR. On the other hand, fortified fillets (BF1 and BF2) presented statistically lower levels of Ca compared with CTR fillets. Steaming significantly decreased Cl (BF1 and BF2) and Ca (CTR and BF1), with TRs ranging from 68% (BF1) to 73% (BF2 and CTR) for Cl and from 60% (BF1) to 65% (CTR and BF2) for Ca. Overall, among all macro elements the lowest TR was observed for Ca in all steamed fillets. . In terms of As (toxic element), fortified gilthead seabream fillets (BF1 and BF2) presented significantly lower contents compared to CTR fillets. Statistically lower TRs were found for macro (Cl, K and Ca), trace (Se, Fe and Zn) and toxic (As) elements in fortified BF1 fish fillets. On the other hand, significantly higher I TRs were found in fortified BF1 fillets after steaming (Table 3).

Fortified common carp also presented significantly higher contents of I and Se (Table 4). Additionally, statistically higher levels of Zn, As (raw and steamed BF1 and BF2) and Fe (only steamed BF2) were found in fortified fillets, compared with non-fortified fish. In contrast, fortified BF2 fillets (raw and steamed) presented significantly lower Ca content compared with the CTR. Fortified fillets (BF1 and BF2) presented significantly lower contents of Cu and Br compared to CTR fillets (raw and steamed). Concerning the steaming effect, in terms of trace elements, steaming significantly increased Fe and Zn contents (CTR and BF2), with TRs above 100% for Fe and around 90% for Zn. In contrast, Cu content significantly decreased after steaming in the CTR (TR of 65%), as well as As content in fortified fillets (BF1 and BF2 with TR of 59% and 62%, respectively). Concerning macro elements, steaming significantly increased Cl (CTR and BF1; TR of 95% and >100%, respectively) and significantly decreased K (CTR and BF2, TR of 68% and 73%, respectively) and Ca (CTR and BF2, TR of 64% and 63%, respectively) contents. Likely to gilthead seabream, among macro elements the lowest TR was observed for Ca. Lower TRs were found for macro (Cl and Ca in BF2), trace (Se and Br in BF2, Fe and Zn in BF1) and toxic (As in BF1) elements in fortified common carp fillets. On the other hand, steamed BF1 fillets revealed higher TRs of Cl, K, Cu and Br, whereas steamed BF2 fillets revealed higher TRs of I, Fe and Zn (Table 4).

Table 3
Concentrations of macro, trace and toxic elements and true retention (TR) of gilthead seabream (*S. aurata*) fed with different experimental diets (CTR - control, BF1 – fortified diet B1, BF2 - fortified diet B2).

	CTR		TR (%)	BF1		TR (%)	BF2		TR (%)
	Raw	Steam		Raw	Steam		Raw	Steam	
Macro elements (mg 100 g^{-1})									
Cl	444 ± 31^{a}	377 ± 8^{A}	73	477 ± 20^{a}	372 ± 5^{A*}	68	530 ± 33^{b}	461 ± 4^{B*}	73
K	1244 ± 24	1444 ± 163	100	1747 ± 371	1596 ± 57	79	1742 ± 150	1683 ± 148	81
Ca	70 ± 19^{c}	52 ± 3^{C*}	65	37 ± 6^{b}	25 ± 1^{B*}	60	12 ± 2^{a}	9.5 ± 0.1^{A}	65
Trace elements (mg kg^{-1})									
I	0.07 ± 0.00^{a}	0.10 ± 0.00^{A*}	125	0.07 ± 0.01^{a}	0.11 ± 0.00^{B*}	134	0.09 ± 0.00^{b}	0.12 ± 0.00^{C*}	110
Se	0.18 ± 0.00^{a}	0.18 ± 0.01^{A}	88	0.23 ± 0.01^{b}	0.23 ± 0.00^{B}	86	0.36 ± 0.01^{c}	0.40 ± 0.00^{C*}	93
Fe	7.1 ± 0.5^{a}	7.8 ± 0.6^{A}	95	12.1 ± 2.8^{b}	9.6 ± 0.6^{A}	69	29.0 ± 2.8^{c}	23.9 ± 3.4^{B*}	69
Cu	2.0 ± 0.0^{b}	<LOD*	n.d.	2.9 ± 0.6c	<LOD*	n.d.	<LOD^{a}	<LOD	n.d.
Zn	1.0 ± 0.2^{a}	1.1 ± 0.0	93	1.6 ± 0.2^{b}	1.3 ± 0.0	71	0.9 ± 0.1^{a}	1.1 ± 0.2	106
Br	3.1 ± 0.2^{b}	<LOD*	n.d.	4.2 ± 0.2^{c}	<LOD*	n.d.	<LOD^{a}	<LOD	n.d.
Toxic elements (mg kg^{-1})									
As	1.8 ± 0.1	1.9 ± 0.1^{B}	91	1.5 ± 0.2	1.5 ± 0.0^{A}	88	1.5 ± 0.1	1.6 ± 0.0^{A}	87

Values are mean ± standard deviation, in wet weight. Different superscript small letters represent statistical differences ($p < 0.05$) between treatments (CTR, BF1, BF2) in raw fish fillets and different superscript capital letters represent statistical differences ($p < 0.05$) between treatments in steamed fish fillets. * represent statistical differences ($p < 0.05$) between raw and steamed fish filets in each treatment. <LOD, below the detection limit.

Table 4
Concentrations of macro, trace and toxic elements and true retention (TR) of common carp (*C. carpio*) fed with different experimental diets (CTR - control, BF1 – fortified diet B1, BF2 - fortified diet B2).

	CTR		TR (%)	BF1		TR (%)	BF2		TR (%)
	Raw	Steam		Raw	Steam		Raw	Steam	
Macro elements (mg 100 g^{-1})									
Cl	92 ± 4	106 ± 5^{B*}	95	101 ± 11	148 ± 3^{C*}	118	84 ± 11	85 ± 9^{A}	81
K	902 ± 57	746 ± 26*	68	918 ± 79	841 ± 51	74	900 ± 30	821 ± 17*	73
Ca	126 ± 9^{b}	98 ± 25^{B*}	64	95 ± 26^{b}	75 ± 5^{B}	64	38 ± 1^{a}	30 ± 2^{A*}	63
Trace elements (mg kg^{-1})									
I	<LOQ^{a}	<LOQ^{A}	n.d.	0.21 ± 0.02^{b}	0.23 ± 0.02^{B}	87	0.19 ± 0.00^{b}	0.21 ± 0.02^{B}	89
Se	0.09 ± 0.00^{a}	0.10 ± 0.01^{A}	87	0.14 ± 0.01^{b}	0.14 ± 0.01^{B}	84	0.14 ± 0.00^{b}	0.14 ± 0.00^{B}	80
Fe	14.7 ± 1.8	20.9 ± 2.5^{A*}	117	20.9 ± 4.6	23.4 ± 1.1^{A}	91	22.1 ± 5.4	35.2 ± 0.5^{B*}	128
Cu	8.0 ± 0.4^{b}	6.3 ± 0.0^{B*}	65	1.8 ± 0.4^{a}	1.6 ± 0.0^{A}	74	2.3 ± 0.5^{a}	2.1 ± 0.1^{A}	73
Zn	11.4 ± 1.2^{a}	12.7 ± 0.4^{A*}	91	13.8 ± 1.6^{b}	14.9 ± 0.9^{B}	87	13.7 ± 0.2^{b}	15.8 ± 0.2^{B*}	93
Br	4.8 ± 0.1^{b}	4.6 ± 0.3^{B}	79	1.9 ± 0.4^{a}	1.9 ± 0.1^{A}	83	2.7 ± 0.4^{a}	2.5 ± 0.0^{A}	73
Toxic elements (mg kg^{-1})									
As	0.08 ± 0.00^{a}	0.07 ± 0.00^{A}	72	0.26 ± 0.02^{b}	0.19 ± 0.01^{B*}	59	0.27 ± 0.01^{b}	0.21 ± 0.00^{B*}	62

Values are mean ± standard deviation, in wet weight. Different superscript small letters represent statistical differences ($p < 0.05$) between treatments (CTR, BF1, BF2) in raw fish fillets and different superscript capital letters represent statistical differences ($p < 0.05$) between treatments in steamed fish fillets. * represent statistical differences ($p < 0.05$) between raw and steamed fish filets in each treatment. n.d., not determined. <LOQ, below the quantification limit.

PCA analysis revealed a notable separation between gilthead seabream and common carp (PC1) related to different elements contents (Fig. 2). In addition, for common carp, two groups were clearly identified according with fish diets, where the first group comprises the fortified fillets (BF1 and BF2) and the second group comprise CTR fillets (PC2). On the other hand, in gilthead seabream, a clear distinction

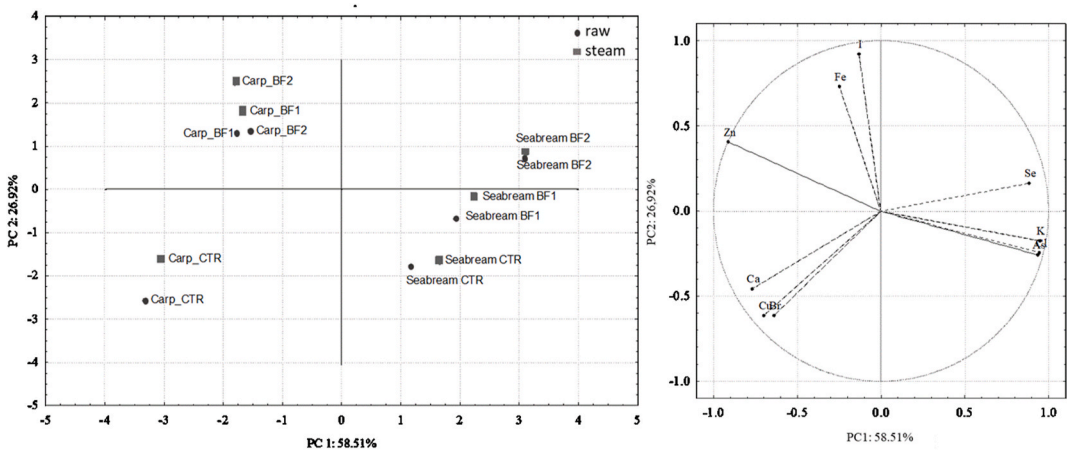

Fig. 2. Score plot of first two principal components (PC1 and PC2) for the nutrients composition in raw and steamed gilthead seabream (*S. aurata*) and common carp (*C. carpio*) fed with different experimental diets. PC1 and PC2 explained 85.43% variance. CTR - control, BF1 - fortified B1, BF2 – fortified B2.

between the most fortified treatment (BF2) from the less fortified treatment (BF1) and CTR was observed (PC2). In terms of culinary treatments, steamed fillets were clearly separated from raw fillets in the CTR and fortified BF2 common carp fillets, whereas no clear separation between raw and steamed fillets was found for seabream in all treatments. Se, Cl, K and As were the main elements influencing the differences between gilthead seabream and common carp. On the other hand, I and Fe were the main elements responsible for the distinction between treatments (CTR, BF1 and BF2). Concerning the culinary treatments in common carp, Cu and Br were the major elements responsible for the separation between raw and steamed CTR fillets, whereas Fe was the major contributor for the separation between raw and steamed BF2 fillets (Fig. 2).

3.2. Nutritional contribution of fortified farmed fish

The consumption of 150 g (adults and pregnant women) and 75 g (children) portion of steamed fortified BF2 gilthead seabream fillets contributed, to higher intakes of I (from 9% for pregnant women to 12% for adults) and Se (from 70% for pregnant women to more than 100% for children) (Table 5). Moreover, steamed BF2 fillets contributed to higher intake of Fe (more than 100% for all population groups), compared to BF1 and CTR fillets. Yet, despite exceeding the daily adequate intake, fortified BF2 fillets contributed to 50% of Se upper intake level (UL) for children and to 8% of Fe UL for adults/pregnant women and 6% of UL for children. On the other hand, the consumption of steamed fortified fillets (BF1 and BF2) contributed to lower intake of Ca (5% and 2% for all population groups, respectively), compared to CTR fillets (11% for adults/pregnant women and 10% for children). The consumption of gilthead seabream fillets (fortified and CTR) also exceeded K daily adequate intake (AI) for children (Table 5). Yet, due to insufficient data, no UL exists for this element (EFSA, 2016).

The consumption of 150 g (adults and pregnant women) and 75 g (children) of steamed fortified common carp fillets (BF1 and BF2) contributed to higher intakes of I (from 16% of AI for pregnant women to 23% for adults) and Se (from 24% for pregnant women to 71% for children), compared to CTR fillets (Table 5). Additionally, the consumption of steamed BF2 fillets contributed to higher intakes of Zn (from 28% for pregnant women to 38% for adults). Contrarily, both fortified BF1 and BF2 fillets contributed to lower intakes of Cu (from 15% % for adults to 23% for children). Despite exceeding the daily AI, fortified BF1 and BF2 fillets contributed, respectively, to 8% and 12% of Fe UL for adults/pregnant women and to 6% and 9% Fe UL for children. In terms of macro elements, the consumption of steamed fortified fillets (BF1 and BF2) contributed to lower intakes of Ca (BF2: 6% for all population groups; BF1: 14% for children and 15% for adults/pregnant women) (Table 5).

4. Discussion

4.1. Effect of steaming on elements content in fortified farmed fish

The incorporation of iodine-rich seaweed (*L. digitata*) and Se-rich yeast in gilthead seabream and common carp feeds resulted in enhanced content of most essential elements, especially I and Se. It is known that culinary treatments, particularly those that require heat, can strongly affect fish nutritional composition depending on the temperature and duration of the cooking process (Barbosa et al., 2018). In line with previous studies (Ribeiro et al., 2015), the results demonstrate that steaming significantly increased I content in gilthead seabream, but not in common carp fillets, compared to raw products. Such results may be explained by the fact that fortified common carp presented lower retention of I after streaming, associated to lower cooking yield (lower ratio of the amount of the edible portion that results from raw products), compared to fortified gilthead seabream. In general, lower cooking yields result from the damaging and solubilisation of higher proportion

Table 5
Nutritional contribution (%) of steamed gilthead seabream (*S. aurata*) and common carp (*C. carpio*) in terms of essential elements in different population groups, considering the consumption of a portion of 150 g of fish for adults and pregnant women, and with the consumption of 75 g of fish for children.

			DRVs (mg day^{-1})[4]	Gilthead seabream			Common carp		
				CTR	BF1	BF2	CTR	BF1	BF2
Macro elements									
Cl	Adults[1]/Pregnant women[2]	AI	3100	18 ± 3	18 ± 0	22 ± 0	5 ± 0	7 ± 0	4 ± 0
	Children[3]	AI	1700	17 ± 3	16 ± 0	20 ± 0	5 ± 0	7 ± 0	4 ± 0
K	Adults[1]	AI	3500	62 ± 7	68 ± 2	72 ± 6	32 ± 1	36 ± 2	35 ± 1
	Pregnant women[2]	AI	4000	54 ± 6	60 ± 2	63 ± 6	28 ± 1	32 ± 2	31 ± 1
	Children[3]	AI	800	>100	>100	>100	70 ± 2	79 ± 5	77 ± 2
Ca	Adults[1]/Pregnant women[2]	AR	750	11 ± 1^{b}	5 ± 0^{a}	2 ± 0^{a}	20 ± 0^{c}	15 ± 1^{b}	6 ± 0^{a}
	Children[3]	AR	390	10 ± 1^{b}	5 ± 0^{a}	2 ± 0^{a}	19 ± 0^{c}	14 ± 1^{b}	6 ± 0^{a}
Trace elements									
I	Adults[1]	AI	0.15	10 ± 0^{a}	11 ± 0^{b}	12 ± 0^{c}	n.d^{a}	23 ± 2^{b}	21 ± 2^{b}
	Pregnant women[2]	AI	0.20	7 ± 0^{a}	8 ± 0^{b}	9 ± 0^{c}	n.d^{a}	17 ± 1^{b}	16 ± 1^{b}
	Children[3]	AI	0.09	8 ± 0^{a}	9 ± 0^{b}	10 ± 0^{c}	n.d^{a}	19 ± 2^{b}	18 ± 1^{b}
Se	Adults[1]	AI	0.07	40 ± 1^{a}	50 ± 1^{b}	85 ± 2^{c}	21 ± 1^{a}	30 ± 2^{b}	30 ± 0^{b}
	Pregnant women[2]	AI	0.085	33 ± 1^{a}	41 ± 0^{b}	70 ± 2^{c}	17 ± 1^{a}	25 ± 1^{b}	24 ± 0^{b}
	Children[3]	AI	0.015	92 ± 3	>100 (29 ± 0)	>100 (50 ± 1)	48 ± 3^{a}	71 ± 4^{b}	69 ± 0^{b}
Fe	Adults[1]	AI	3.4	31 ± 2^{a}	54 ± 12^{a}	>100 (8 ± 1)^{b}	92 ± 10	>100 (8 ± 0)	>100 (12 ± 0)
	Pregnant women[2]	AI	2.9	37 ± 3^{a}	63 ± 15^{a}	>100 (8 ± 1)^{b}	>100 (7 ± 1)	>100 (8 ± 0)	>100 (12 ± 0)
	Children[3]	AI	0.6	98 ± 8	>100 (2 ± 0)	>100 (6 ± 1)	>100 (5 ± 1)	>100 (6 ± 0)	>100 (9 ± 0)
Cu	Adults[1]	AI	1.6	n.d.	n.d.	n.d.	59 ± 0^{b}	15 ± 0^{a}	20 ± 1^{a}
	Pregnant women[2]	AI	1.5	n.d.	n.d.	n.d.	63 ± 0^{b}	16 ± 0^{a}	21 ± 1^{a}
	Children[3]	AI	0.7	n.d.	n.d.	n.d.	68 ± 0^{b}	18 ± 0^{a}	23 ± 1^{a}
Zn	Adults[1]	AR	6.2	3 ± 0	3 ± 0	3 ± 1	31 ± 1^{a}	36 ± 2^{ab}	38 ± 1^{b}
	Pregnant women[2]	AR	8.6	2 ± 0	2 ± 0	2 ± 0	22 ± 1^{a}	26 ± 2^{ab}	28 ± 0^{b}
	Children[3]	AR	3.6	2 ± 0	3 ± 0	2 ± 0	26 ± 1^{a}	31 ± 2^{ab}	33 ± 0^{b}

Values are mean ± standard deviation. The Nutritional contribution (NC; %) are presented for [1]adults (>18 years) with mean body weight in Europe (70 kg), [2]children (1–3 years) with mean body weight in Europe (13 kg) and [3]pregnant/lactating women with mean body weights in Europe (67 kg) set by EFSA (2012). [4]The Dietary Reference Values (DRVs) are presented as Adequate Intakes (AI) for I (EFSA, 2014a), Se (EFSA, 2014b), Fe (EFSA, 2015b), Cu (EFSA, 2015c), Cl (EFSA, 2019) and K (EFSA, 2016), as well as the tolerable upper intake level (UL; in parenthesis) and adequate requirement (AR) for Ca (EFSA, 2015d) and Zn (EFSA, 2014c). n.d., not determined due to contents bellow the detection limit (<LOD). No tolerable upper intake level (UL) has been set for K by EFSA due to insufficient data (EFSA, 2016). Different superscript small letters represent statistical differences ($p < 0.05$) between treatments (CTR, BF1, BF2). CTR – control; BF1 – fortified B1; BF2 – fortified B2.

of musculature connective tissue and dehydration of the muscle fibrils (Oliveira et al., 2019). Interestingly, increased I content was also previously reported in steamed anchovy and whiting, which presented lower contents in raw, compared to decreased I content in steamed horse mackerel, bluefish, Atlantic bonito and striped red mullet, which presented higher contents in raw meat (Erkan, 2011). Similarly, steaming increased Se content in most fortified gilthead seabream fillets (BF2), but not in fortified common carp. Increased Se content was previously reported in blue shark after grilling and steaming, which is associated to water loss during culinary treatment (Matos et al., 2015); whereas no statistically significant differences between Se content after cooking were reported in sardine, mackerel, hake and scabbardfish (Martins et al., 2011). Previous authors explained that I and Se are mainly bound to proteins (Hou, 2009; Vicente-Zurdo et al., 2019) and, therefore less prone to leaching during mild cooking procedures, such as steaming. In general, gilthead seabream presents higher protein and fat contents, whereas common carp presents higher moisture contents (Huss, 1995). Additionally, increased I and Se contents after fish cooking have been associated with the concentration of these elements due to water losses (Alves et al., 2018; Erkan, 2011; Martins et al., 2011). Nevertheless, the present study demonstrated that steaming has no detrimental effect in enhanced I and Se contents in fortified fish fillets from both species.

In terms of other essential nutrients contents, steaming resulted in increased Cl, Fe and Zn in common carp fillets, but not in gilthead seabream. Increased Fe and Zn contents has been previously reported in fried gilthead seabream (Mnari et al., 2012), whereas steaming resulted in increased Zn content in plaice, mackerel and hake (Alves et al., 2018). In contrast, steaming resulted in decreased Cl and Fe contents in fortified gilthead seabream fillets, as well as Cu and Br contents in both fortified and non-fortified fillets. On the other hand, steaming resulted in decreased As content in fortified common carp fillets, Cu content in non-fortified fillets and K content in both fortified and non-fortified fillets. Interestingly, Ca content decreased after steaming in fortified and non-fortified fillets from both species. During thermal processing, the solubilisation of some minerals, such as the divalent elements, may occur due to muscle proteins denaturation (Kong et al., 2007; Mohan et al., 2008). The denaturation of sarcoplasmic and myofibrillar proteins results in the disconnection and dehydration of the muscle fibrils, leading to protein structural changes and decreased stability to form complexes protein-mineral complexes, and to consequent solubilisation of some minerals, such as Ca and Mg, intrinsically linked to fish muscle sarcoplasmic and myofibrillar proteins (Bastías et al., 2017; Ochiai and Ozawa, 2020). Previous studies also reported different changes in elements content, likely related with fish species and the different culinary treatments used. For example, boiling resulted in increased Ca content (Karimian-Khosroshahi et al., 2016), as well as decreased Zn and K contents (Gokoglu et al., 2004) in rainbow trout, as well decreased Ca, K, Fe and Zn contents in gilthead seabream (Mnari et al., 2012) and decreased K content in kutum roach (Hosseini et al., 2014). Furthermore, decreased contents of K and Zn were observed in grilled gilthead seabream (Mnari et al., 2012) and rainbow trout (Gokoglu et al., 2004), respectively, while increased content of K was observed in African catfish (Ersoy and Özeren, 2009) and rainbow trout (Gokoglu et al., 2004) after grilling. Frying increased Cu content in kutum roach (Hosseini et al., 2014), Cu and Ca content in rainbow trout (Gokoglu et al., 2004; Karimian-Khosroshahi et al., 2016), whereas decreased contents of Ca and Zn in fried rainbow trout (Gokoglu et al., 2004). Microwaving increased K content in rainbow trout (Gokoglu et al., 2004; Karimian-Khosroshahi et al., 2016), as well as K and Ca contents in African catfish (Ersoy and Özeren, 2009). Contrarily, increased content of K and decreased content of Fe were reported in kutum roach after microwave cooking (Hosseini et al., 2014). Furthermore, decreased content of Ca, K, Fe and Zn was also reported in gilthead seabream after oven-cooking (Mnari et al., 2012). Both losses and concentrations of macro and trace elements are mainly associated to water loss, as result of the evaporation, dehydration of muscle fibrils, and probably to some heat-induced protein denaturation during steaming, leading to minerals leaching from water protein structures or by the concentration of minerals due to weight loss (Oliveira et al., 2019; Sobral et al., 2018). The present results contribute with relevant data, highlighting that the elemental composition is closely related to cooking procedures, as well as to the initial elemental content in raw fish and therefore being species-specific, as reported in the literature (Mnari et al., 2012; Petricorena, 2015). In fact, comparing the elemental composition between each treatment (CTR, BF1 and BF2), a different pattern was observed for each species with results showing a clear distinction between common carp and gilthead seabream. Moreover, the different fortification strategies contributed to distinct effects on fish elemental composition, whereas the steam cooking treatment seems to have less influence on fillets elemental composition, especially in gilthead seabream. However, other factors related to species-specific may also influence the different elemental profiles. For example, Ribeiro et al. (201%) reported increased I content after steaming in gilthead seabream fish with similarly final body weight (488–506 g compared to 491–525 g from the present study), despite the different origin (i.e. farmed in different aquaculture stations). In contrast, Mnari et al. (2012) reported increased Fe and Zn contents in wild and farmed gilthead seabream with lower body weight (71 $\pm$ 1 g and 85 $\pm$ 2 g, respectively), compared to the present study (decreased Fe and Zn in farmed gilthead seabream with 549–525 g of body weight). Additionally, considering different species and different origins, but specimens' similar sizes different patterns in minerals contents was observed. For example, with similar body weight (1–1.3 kg), rainbow trout specimens (Karimian-Khosroshahi et al., 2016) and katum rach specimens (Hosseini et al., 2014), from different origins presented increased Ca content after cooking whereas decreased Ca content was observed in steamed common carp, suggesting that fish elemental composition is also dependent on specimens' origins and sizes.

The nutrients true retention (TR) is an important method for the determination of nutrients content in cooked foods, considering changes in weight and nutrient composition during cooking (Bognár and Piekarski, 2000). Most macro and trace elements TR values, with the exception of Ca, were approximately in the same range to those estimated and found by Bógnar (2002), reflecting the differences associated to specific cooking yields. Noteworthy, TR values nearly 100% indicate that the nutrient is less prone to leaching or degradation process during cooking (Badiani et al., 2013), which is the case of most trace elements. Moreover, in line with previous studies, Ca was the least retained element in both gilthead seabream and common carp (Badiani et al., 2013), showing to be the element with higher losses during culinary procedures. Fortified gilthead seabream fillets (BF2) revealed the highest TRs for most trace elements (I, Se, Zn), combined with the lowest TR of toxic element (As). Similarly, fortified common carp fillets (BF1), revealed higher TRs of macro (Cl, K) and trace elements (I, Se, Fe, Cu and Br), with the lowest TR for the toxic element (As), demonstrating that steaming affected differentially the elements content with potentially added value to fortified fish products.

4.2. *Fortified farmed fish improve nutritional benefits to human health*

The consumption of a usual portion of 150 g of steamed fortified gilthead seabream for adults and pregnant women and 75 g for children contributes to increased NC of macro (Cl and K), and trace (Se and Fe) elements. Similarly, the consumption of 150 g of fortified common carp also improved the NC of macro (Cl and K) and trace (I, Se, Fe and Zn) elements. A previous study assessed the nutritional value of gilthead seabream fortified with *L. digitata* and found that the consumption of 160 g of steamed seabream fillet covered about 80% of I daily AI for adults (Ribeiro et al., 2015). Yet, it is worth mentioning that *L. digitata* was supplied at much higher levels (i.e. nine times more). Increased NC of I (12.4% of AI for adults) and Se (97.8% of AI for adults) was also reported in rainbow trout fillets fortified with I-rich seaweed (*L. digitata*)

and Se-rich yeast (Ribeiro et al., 2017). In comparison, fortified rainbow trout fillets showed higher NC of Se (+12.8%) and Zn (+2.1%) and lower NC of K (−55.8%) and Fe (−100%) than fortified gilthead seabream, as well as higher NC of Se (+67.8%) and lower NC of I (−10.6%), K (−19.8%), Fe (−100%) and Zn (−30.9%) than fortified common carp. Although, it is worth mentioning that in the previous study the nutritional value was assessed in raw rainbow trout fillets and that I-rich seaweed and Se-rich yeast were incorporated at different levels from the present study (0.365% of *L. digitata* and 1% of Se-rich yeast in the previous study, against 0.8% of *L. digitata* and 0.035% of Se-rich yeast in gilthead seabream and 0.54% of *L. digitata* and 0.1% of Se-rich yeast in common carp). To the author's knowledge, no studies addressed the health nutritional value of fortified fish fillets considering the effect of culinary procedures in a wide range of essential nutrients. Only the influence of steaming to the levels of essential and toxic elements was assessed in several fish species available in European markets (Alves et al., 2018). Considering the results from this study, higher NC of Se and Fe is achieved by the consumption of fortified gilthead seabream and fortified common carp relatively to five fish species (plaice, hake, tuna, mackerel and monkfish; Alves et al., 2018). Additionally, comparing to the previous study of Alves et al. (2018), fortified common carp contributed to higher NC of I relatively to hake and mackerel, and of Zn comparing to hake, tuna, mackerel, plaice and monkfish.

The present results clearly demonstrate that fortification strategies with iodine-rich seaweed (*L. digitata*) and Se-rich yeast in gilthead seabream contributes to reduce Se and Fe deficiencies in target population groups. In contrast, fortified common carp contributes to reduce I and Fe deficiencies of consumers. Despite the benefits of fortification strategies used in this study outweigh the apparent risks, since increased intakes of I and Se offer added value for consumers' diets, parsimonious consumption of common carp should be considered particularly for children to avoid exceeding the UL set for Se. Additionally, particular attention should be given to fortification strategies of both species to avoid exceeding ULs set for Fe to all population groups.

5. Conclusions

This study provides new insights into the effect of steaming in nutritional enrichment of fortified gilthead seabream and common carp fillets. The dietary strategies assessed through the supplementation with I-rich macroalgae and Se-rich yeast, revealed to be highly efficient in gilthead seabream Se fortification (more than 90% increase) and in common carp iodine fortification (more than 100% increase). Results clearly indicate that steaming can indeed affect macro, trace and toxic elements contents, being strongly related with the chemical properties of each element and fish species. Steaming resulted in significant increased contents of I and Se in fortified gilthead seabream fillets, as well as in significant decreased contents of Cl, Fe, Cu and Br. On the other hand, steaming resulted in significant increased contents of Fe, Zn and Cl in fortified common carp fillets, as well as in significant decreases in K and As contents. In both fortified fish species, steaming significantly decreased Ca content. Additionally, the main essential elements (I, Se and Fe) NC were improved with fortified fish fillets. Yet, whereas I nutritional contribution could still be further improved, particular attention should be given to Fe and Se nutritional contribution to avoid exceeding the current recommendations. The findings of the present study clearly demonstrate the great potential of the studied fortification strategies to reduce essential elements deficiencies in consumers, especially those associated with I, Se and Fe, and the related adverse disorders/diseases. Moreover, fish fortification seems to be an excellent strategy to enhance the nutritional quality of farmed fish products, and steaming can be considered as a suitable cooking procedure for a healthy consumption. Nonetheless, future studies regarding elements bioaccessibility and bioavailability of fortified fish will provide more insights for the realistic assessment on nutritional benefits to human health of fortification strategies with natural ingredients from sustainable sources.

CRediT authorship contribution statement

Vera Barbosa: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Ana Luísa Maulvault:** Investigation, Writing – review & editing. **Patrícia Anacleto:** Investigation, Writing – review & editing. **Marta Santos:** Investigation. **Mónica Mai:** Investigation. **Helena Oliveira:** Investigation, Writing – review & editing. **Inês Delgado:** Investigation. **Inês Coelho:** Investigation. **Marisa Barata:** Investigation. **Ravi Araújo-Luna:** Investigation. **Laura Ribeiro:** Investigation. **Piotr Eljasik:** Investigation. **Małgorzata Sobczak:** Investigation. **Jacek Sadowski:** Investigation. **Agnieszka Tórz:** Investigation. **Remigiusz Panicz:** Investigation. **Jorge Dias:** Investigation. **Pedro Pousão-Ferreira:** Resources. **Maria Luísa Carvalho:** Resources, Writing – review & editing. **Marta Martins:** Writing – review & editing. **António Marques:** Conceptualization, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.2021.112218.

Ethical statement

Fish trials were conducted according to legal regulations (EU Directive, 2010/63) and approved by the Ethical Committee of the EPPO-IPMA and ZUT, overseen by the National Competence Authority. All researchers and technicians involved in the maintenance, handling and sampling of live animals were certified in Laboratory Animal Sciences, by the Federation of European Laboratory Animal Science Associations (FELASA).

References

Alves, R.N., Maulvault, A.L., Barbosa, V.L., Fernandez-Tejedor, M., Tediosi, A., Kotterman, M., van den Heuvel, F.H.M., Robbens, J., Fernandes, J.O., Romme Rasmussen, R., Sloth, J.J., Marques, A., 2018. Oral bioaccessibility of toxic and essential elements in raw and cooked commercial seafood species available in European markets. Food Chem. 267, 15–27. https://doi.org/10.1016/j.foodchem.2017.11.045.

Badiani, A., Stipa, S., Bitossi, F., Pirini, M., Bonaldo, A., Gatta, P.P., Rotolo, M., Testi, S., 2013. True retention of nutrients upon household cooking of farmed portion-size European sea bass (*Dicentrarchus labrax L.*). LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 50 (1), 72–77. https://doi.org/10.1016/j.lwt.2012.06.026.

Barbosa, V., Maulvault, A.L., Alves, R.N., Kwadijk, C., Kotterman, M., Tediosi, A., Fernández-tejedor, M., Sloth, J.J., Granby, K., Rasmussen, R.R., Robbens, J., Witte, B. De, Trabalón, L., Fernandes, J.O., Cunha, S.C., Marques, A., 2018. Effects of steaming on contaminants of emerging concern levels in seafood. Food Chem. Toxicol. 118, 490–504. https://doi.org/10.1016/j.fct.2018.05.047.

Barbosa, V., Maulvault, A.L., Anacleto, P., Santos, M., Mai, M., Oliveira, H., Delgado, I., Coelho, I., Barata, M., Araújo-Luna, R., Ribeiro, L., Eljasik, P., Sobczak, M., Sadowski, J., Tórz, A., Panicz, R., Dias, J., Pousão-Ferreira, P., Carvalho, M.L., Martins, M., Marques, A., 2020. Enriched feeds with iodine and selenium from natural and sustainable sources to modulate farmed gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) fillets elemental nutritional value. Food Chem. Toxicol. 140, 111330. https://doi.org/10.1016/J.FCT.2020.111330.

Bastías, J.M., Balladares, P., Acuña, S., Quevedo, R., Muñoz, O., 2017. Determining the effect of different cooking methods on the nutritional composition of salmon (*Salmo salar*) and chilean jack mackerel (*Trachurus murphyi*) fillets. PloS One 12 (7), e0180993. https://doi.org/10.1371/journal.pone.0180993.

Bevis, L.E.M., 2015. Soil-to-Human mineral transmission with an emphasis on zinc, selenium, and iodine. Springer Sci. Rev. 3, 77–96. https://doi.org/10.1007/s40362-014-0026-y.

Bógnar, A., 2002. Tables on weight yield of food and retention factors of food constituents for the calculation of nutrient composition of cooked foods (dishes); BFE-R-02-03; berichte der Bundesforschungsanstalt für Ernährung: karlsruhe, Germany, 2002. http://www.fao.org/uploads/media/bognar_bfe-r-02-03.pdf. (Accessed 20 December 2020).

Bognár, A., Piekarski, J., 2000. Guidelines for recipe information and calculation of nutrient composition of prepared foods (Dishes). J. Food Compos. Anal. 13, 391–410. https://doi.org/10.1006/jfca.2000.0922.

Coelho, I., Rego, A., Gueifão, S., Nascimento, A.C., Castanheira, I., 2017. Application of chemometric methods for multi-elemental characterization of fruit juices and nectars analysed in the Portuguese Total Diet Studies pilot study. J. Chemometr. 31 (2), e2868. https://doi.org/10.1002/cem.2868.

Cotter, P.A., McLean, E., Craig, S.R., 2009. Designing fish for improved human health status. Nutr. Health (Bicester) 20 (1), 1–9. https://doi.org/10.1177/026010600902000101.

Delgado, I., Ventura, M., Gueifão, S., Coelho, I., Nascimento, A.C., Silva, J.A.L., Castanheira, I., 2019. 12th IFDC 2017 special issue – iodine, selenium and iron contents in Portuguese key foods as consumed. J. Food Compos. Anal. 79, 39–46. https://doi.org/10.1016/j.jfca.2019.03.004.

EFSA, 2009. EFSA panel on contaminants in the food chain (CONTAM); scientific opinion on arsenic in food. 2009. EFSA J. 7 (10) https://doi.org/10.2903/j.efsa.2009.1351, 1351, 199.

EFSA, 2012. Scientific Committee; Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. 2012. EFSA J. 10 (3), 2579. 32. http://doi:10.2903/j.efsa.2012.2579.

EFSA, 2014a. Panel on dietetic products nutrition and allergies (NDA); scientific opinion on dietary reference values for iodine. 2014. EFSA J. 12 (5) https://doi.org/10.2903/j.efsa.2014.3660, 3660, 57.

EFSA, 2014b. Panel on dietetic products nutrition and allergies (NDA); scientific opinion on dietary reference values for selenium. 2014. EFSA J. 12 (10) https://doi.org/10.2903/j.efsa.2014.3846, 3846, 67.

EFSA, 2014c. Panel on dietetic products nutrition and allergies (NDA); scientific opinion on dietary reference values for zinc. 2014. EFSA J. 12 (10), 3844. 3876. http://doi:10.2903/j.efsa.2014.3844.

EFSA, 2015a. EFSA panel on contaminants in the food chain (CONTAM); Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. 2015. EFSA J. 13, 1–36. https://doi.org/10.2903/j.efsa.2015.3982.

EFSA, 2015b. Panel on dietetic products, nutrition and allergies (NDA); scientific opinion on dietary reference values for iron. 2015. EFSA J. 13 (10), 4254. 115. https://doi:10.2903/j.efsa.2015.4254.

EFSA, 2015c. Panel on dietetic products, nutrition and allergies (NDA); scientific opinion on dietary reference values for copper. 2015. EFSA J. 13 (10), 4253. 51. https://doi:10.2903/j.efsa.2015.4253.

EFSA, 2015d. Panel on dietetic products, nutrition and allergies (NDA); scientific opinion on dietary reference values for calcium. 2015. EFSA J. 13 (5), 4101. 4182. http://doi:10.2903/j.efsa.2015.4101.

EFSA, 2016. Panel on dietetic products, nutrition and allergies (NDA); scientific opinion on dietary reference values for potassium. 2016. EFSA J. 14 (10), 4592. 56. https://doi:10.2903/j.efsa.2016.4592.

EFSA, 2019. Panel on panel on nutrition, novel foods and food allergens (NDA); scientific opinion on dietary reference values for chloride. 2019. EFSA J. 17 (9) https://doi.org/10.2903/j.efsa.2019.5779, 5779, 24.

Erkan, N., 2011. Iodine content of cooked and processed fish in Turkey. Int. J. Food Sci. Technol. 46 (8), 1734–1738. https://doi.org/10.1111/j.1365-2621.2011.02684.x.

Ersoy, B., Özeren, A., 2009. The effect of cooking methods on mineral and vitamin contents of African catfish. Food Chem. 115 (2), 419–422. https://doi.org/10.1016/j.foodchem.2008.12.018.

EUMOFA, 2019. European Market Observatory for Fisheries and Aquaculture Products. the Eu Fish Market. https://doi.org/10.2771/168390, 2019 Edition.

European Commission, 2007. Commission Recommendation of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (2007/526/EC). Off. J. Eur. Union L197, 1–89. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:197:0001:0089:EN:PDF. (Accessed 10 January 2021).

European Committee for Standardization, 2007. European Standard EN 15111:2007. Foodstuffs—Determination of Trace Elements—Determination of Iodine by ICP-MS (Inductively Coupled Plasma Mass Spectrometry); CEN:Brussels, Belgium, 2007.

European Committee for Standardization, 2009. European Standard EN 15763:2009. Foodstuffs—Determination of Trace Elements—Determination of Arsenic, Cadmium, Mercury and Lead in Foodstuffs by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after Pressure Digestion. CEN, Brussels, Belgium, 2009.

European Union, 2019. Agriculture, forestry and fishery statistics, Eurostat, 2019 Edition. https://ec.europa.eu/eurostat/documents/3217494/10317767/KS-FK-19-001-EN-N.pdf/742d3fd2-961e-68c1-47d0-11cf30b11489?t=1576657490000. (Accessed 10 January 2021).

FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainable Development Goals. Food and Agriculture Organization of the United Nations, Rome. https://doi.org/10.1093/japr/3.1.101.

Gokoglu, N., Yerlikaya, P., Cengiz, E., 2004. Effects of cooking methods on the proximate composition and mineral contents of rainbow trout (*Oncorhynchus mykiss*). Food Chem. 84 (1), 19–22. https://doi.org/10.1016/S0308-8146(03)00161-4.

Hasler, C.M., 2002. Functional foods: benefits, concerns and challenges — a position paper from the American Council on science and health. J. Nutr. 132 (12), 3772–3781. https://doi.org/10.1093/jn/132.12.3772.

Hosseini, H., Mahmoudzadeh, M., Rezaei, M., Mahmoudzadeh, L., Khaksar, R., Khosroshahi, N.K., Babakhani, A., 2014. Effect of different cooking methods on minerals, vitamins and nutritional quality indices of kutum roach (*Rutilus frisii kutum*). Food Chem. 148, 86–91. https://doi.org/10.1016/j.foodchem.2013.10.012.

Hou, X., 2009. Iodine speciation in foodstuffs, tissues, and environmental samples: iodine species and analytical method. In: Preedy, Victor R., Burrow, Gerard N., Watson, Ronald (Eds.), Comprehensive Handbook of Iodine. Academic Press, pp. 139–150. https://doi.org/10.1016/B978-0-12-374135-6.00015-7.

Huss, H.H., 1995. Quality and Quality Changes in Fresh Fish. Fisheries Technical Paper No. 348. FAO, Rome, Italy. http://www.fao.org/3/V7180E/V7180E00.htm. (Accessed 20 December 2020).

Karimian-Khosroshahi, N., Hosseini, H., Rezaei, M., Khaksar, R., Mahmoudzadeh, M., 2016. Effect of different cooking methods on minerals, vitamins, and nutritional quality indices of rainbow trout (*Oncorhynchus mykiss*). Int. J. Food Prop. 19, 2471–2480. https://doi.org/10.1080/10942912.2015.1039028.

Kong, F., Tang, J., Rasco, B., Crapo, C., 2007. Kinetics of salmon quality changes during thermal processing. J. Food Eng. 83 (4), 510–520. https://doi.org/10.1016/j.jfoodeng.2007.04.002.

Kongkachuichai, R., Napatthalung, P., Charoensiri, R., 2002. Heme and nonheme iron content of animal products commonly consumed in Thailand. J. Food Compos. Anal. 15 (4), 389–398. https://doi.org/10.1006/jfca.2002.1080.

Luten, J., Schram, E., Elvevoll, E., 2008. Tailor-made functional seafood for consumers: dietary modulation of selenium and taurine in farmed fish. In: Lie, Øyvind (Ed.), Improving Farmed Fish Quality and Safety, Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, pp. 343–362. https://doi.org/10.1533/9781845694920.2.343.

Martins, C.T., Almeida, C.M.M., Alvito, P.C., 2011. Selenium content of raw and cooked marine species consumed in Portugal. Food Analyt. Methods 4, 77–83. https://doi.org/10.1007/s12161-009-9119-7.

Matos, J., Lourenço, H.M., Brito, P., Maulvault, A.L., Martins, L.L., Afonso, C., 2015. Influence of bioaccessibility of total mercury, methyl-mercury and selenium on the risk/benefit associated to the consumption of raw and cooked blue shark (*Prionace glauca*). Environ. Res. 143, 123–129. https://doi.org/10.1016/j.envres.2015.09.015.

Maulvault, A.L., Anacleto, P., Machado, R., Amaral, A., Carvalho, M.L., Lourenço, H.M., Nunes, M.L., Marques, A., 2012. Effect of sex, maturation stage and cooking methods on the nutritional quality and safety of black scabbard fish (*Aphanopus carbo Lowe*, 1839). J. Sci. Food Agric. 92 (7), 1545–1553. https://doi.org/10.1002/jsfa.4741.

Maulvault, A.L., Cardoso, C., Nunes, M.L., Marques, A., 2013. Risk-benefit assessment of cooked seafood: black scabbard fish (*Aphanopus carbo*) and edible crab (*Cancer pagurus*) as case studies. Food Contr. 32 (2), 518–524. https://doi.org/10.1016/j.foodcont.2013.01.026.

Mnari, B.A., Zaghbib, S.F., Dhibi, M., Harzallah, H.J., Dabbou, S., El Cafsi, M., Hammami, M., Chaouch, A., 2012. Effects of different cooking methods on the mineral contents of wild and farmed sea bream (*Sparus aurata*). Int. J. Food Sci. Technol. 47 (9), 1964–1969. https://doi.org/10.1111/j.1365-2621.2012.03057.x.

Mohan, M., Ramachandran, D., Sankar, T.V., Anandan, R., 2008. Physicochemical characterization of muscle proteins from different regions of mackerel (*Rastrelliger kanagurta*). Food Chem. 106 (2), 451–457. https://doi.org/10.1016/j.foodchem.2007.05.024.

Ochini, Y., Ozawa, H., 2020. Biochemical and physicochemical characteristics of the major muscle proteins from fish and shellfish. Fish. Sci. 86, 729–740. https://doi.org/10.1007/s12562-020-01444-y.

Oliveira, H., Muniz, J.A., Bandarra, N.M., Castanheira, I., Coelho, I.R., Delgado, I., Gonçalves, S., Lourenço, H.M., Motta, C., Duarte, M.P., Nunes, M.L., Gonçalves, A., 2019. Effects of industrial boiling on the nutritional profile of common octopus (*Octopus vulgaris*). Foods 8 (9), 1–15. https://doi.org/10.3390/foods8090411.

Petricorena, Z.C., 2015. Chemical composition of fish and fishery products. In: Cheung, P. (Ed.), Handbook of Food Chemistry. Springer, Berlin, Heidelberg, pp. 1–28. https://doi.org/10.1007/978-3-642-41609-5_12-1.

Pinkaew, S., Karrila, T.T., 2015. Key properties of iodine-, iron- and zinc- fortified fish cracker: effects of ambient shelf storage on iodine retention and quality indicators. Int. J. Food Sci. Technol. 50 (9), 1979–1987. https://doi.org/10.1111/ijfs.12871.

Ramos, A., Bandarra, N.M., Rema, P., Vaz-Pires, P., Nunes, M.L., Andrade, A.M., Cordeiro, A.R., Valente, L.M.P., 2008. Time course deposition of conjugated linoleic acid in market size rainbow trout (*Oncorhynchus mykiss*) muscle. Aquaculture 274 (2–4), 366–374. https://doi.org/10.1016/j.aquaculture.2007.11.040.

Reboredo, F.H., Barbosa, A., Silva, M.M., Carvalho, M.L., Santos, J.P., Pessoa, M.F., Lindon, F., Ramalho, J.C., Guerra, M., 2020. Mineral content of food supplements of plant origin, by energy dispersive X-ray fluorescence: a risk assessment. Expos. Health 12, 917–927. https://doi.org/10.1007/s12403-020-00354-9.

Ribeiro, A.R., Gonçalves, A., Colen, R., Nunes, M.L., Dinis, M.T., Dias, J., 2015. Dietary macroalgae is a natural and effective tool to fortify gilthead seabream fillets with iodine: effects on growth, sensory quality and nutritional value. Aquaculture 437, 51–59. https://doi.org/10.1016/j.aquaculture.2014.11.028.

Ribeiro, A.R., Gonçalves, A., Bandarra, N., Nunes, M.L., Dinis, M.T., Dias, J., Rema, P., 2017. Natural fortification of trout with dietary macroalgae and selenised-yeast increases the nutritional contribution in iodine and selenium. Food Res. Int. 99, 1103–1109. https://doi.org/10.1016/j.foodres.2016.10.030.

Ribeiro, A.R., Altintzoglou, T., Mendes, J., Nunes, M.L., Dinis, M.T., Dias, J., 2019. Farmed fish as a functional food: perception of fish fortification and the influence of origin – insights from Portugal. Aquaculture 501, 22–31. https://doi.org/10.1016/j.aquaculture.2018.11.002.

Saltzman, A., Birol, E., Bouis, H.E., Boy, E., De Moura, F.F., Islam, Y., Pfeiffer, W.H., 2013. Biofortification: progress toward a more nourishing future. Glob. Food Secur. 2 (1), 9–17. https://doi.org/10.1016/j.gfs.2012.12.003.

Sidari, R., Tofalo, R., 2019. A comprehensive overview on microalgal-fortified/based food and beverages. Food Rev. Int. 35 (8), 778–805. https://doi.org/10.1080/87559129.2019.1608557.

Sobral, M.M.C., Cunha, S.C., Faria, M.A., Ferreira, I.M., 2018. Domestic cooking of muscle Foods : impact on composition of nutrients and contaminants. Compr. Rev. Food Sci. Food Saf. 17 (2), 309–333. https://doi.org/10.1111/1541-4337.12327.

Tontisirin, K., Nantel, G., Bhattacharjee, L., 2002. Food-based strategies to meet the challenges of micronutrient malnutrition in the developing world. Proc. Nutr. Soc. 61 (2), 243–250. https://doi.org/10.1079/pns2002155.

United Nations, 2020. The Sustainable Development Goals Report 2020, the Sustainable Development Goals. United Nations Publications. https://doi.org/10.29171/azu_acku_pamphlet_k3240_s878_2016. (Accessed 18 December 2020).

USDA, 2007. Composition of Foods Raw, Processed, Prepared, vol. 27. USDA National Nutrient Database for Standard Reference. http://www.ars.usda.gov/ba/bhnrc/ndl. (Accessed 1 December 2020).

Valente, L.M.P., Rema, P., Ferraro, V., Pintado, M., Sousa-Pinto, I., Cunha, L.M., Oliveira, M.B.B., Araújo, M., 2015. Iodine enrichment of rainbow trout flesh by dietary supplementation with the red seaweed *Gracilaria vermiculophylla*. Aquaculture 446, 132–139. https://doi.org/10.1016/j.aquaculture.2015.05.004.

Vicente-Zurdo, D., Gómez-Gómez, B., Pérez-Corona, M.T., Madrid, Y., 2019. Impact of fish growing conditions and cooking methods on selenium species in swordfish and salmon fillets. J. Food Compos. Anal. 83, 103275. https://doi.org/10.1016/j.jfca.2019.103275.

WHO, 2013. Salt Reduction and Iodine Fortification Strategies in Public Health; Report of a Joint Technical Meeting Convened by the World Health Organization and the George Institute for Global Health in Collaboration with the International Council for the Control of Iodine Deficiency Disorders Global Network. World Health Organization WHO, Sydney, Australia, 2013. https://apps.who.int/iris/bitstream/handle/10665/101509/9789241506694_eng.pdf. (Accessed 18 December 2020).