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In vitro bioaccessibility of macro and trace elements in biofortified and conventional farmed gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*)

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ABSTRACT

Biofortification is a promising strategy to improve the nutrient profile of farmed fish but requires consideration of the nutrient bioaccessible fraction. In this study, the *in vitro* bioaccessibility of macro and trace elements was investigated in biofortified and conventional farmed gilthead seabream and common carp, also taking into account the effect of cooking (by steaming). Biofortification enhanced iodine and selenium levels in seabream and carp fillets. Steaming increased iodine and selenium contents in biofortified seabream, and increased selenium and decreased copper levels in biofortified carp. Higher iodine bioaccessibilty (> 80%) was observed in biofortified seabream compared to biofortified carp (45%). In both species, selenium, iron, and zinc bioaccessibility was ≥ 70%. Upon steaming iodine and iron bioaccessibility decreased in seabream, while selenium bioaccessibility decreased in carp. The consumption of steamed biofortified seabream and carp contributes to significantly higher daily intakes of iodine (up to 12% and 10%, respectively) and selenium (up to 54% and above 100%, respectively) compared to conventional counterparts. The present study demonstrates the potential of developing innovative biofortified farmed fish using natural sustainable feed ingredients to improve the intake of important nutrients for human health.

1. Introduction

Seafood-based diets are associated to health benefits in view of their being a valuable source of essential nutrients, such as long chain polyunsaturated n-3 fatty acids, vitamin D, selenium, and iodine (EFSA, 2015a; Guérin et al., 2011). A regular and balanced consumption of seafood may overcome widespread nutritional deficiencies and is recommended during pregnancy for the positive impact on functional outcomes of children's neurodevelopment and in adulthood to lower the risk of cardiovascular diseases (EFSA, 2014; FAO/WHO, 2011).

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However, one third of the world's population still suffers from nutritional deficiencies, particularly of iron, iodine, selenium, zinc, and vitamin A, which are nutrients related with impaired neurophysiological and immunological functions during the most crucial stages of human growth (FAO, 2020; FAO et al., 2021). Significant efforts have been put into finding alternative resources to reduce hunger, improve food security, nutrition and promote food systems sustainability (Bellia et al., 2021; FAO, 2020). Aquaculture offers the opportunity to produce seafood with additional health benefits by tailoring the nutritional contents of farmed species through the incorporation of functional components in feeds (Barbosa et al., 2020; Bellia et al., 2021; Ferreira et al., 2020; Larsen et al., 2011; Ramalho Ribeiro et al., 2017; Valente et al., 2015). Successful biofortification (i.e., improvement of the nutritional profile of fish using diets supplemented with natural ingredients) has been achieved in farmed gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) through the incorporation of I-rich seaweed (*Laminaria digitata*) and Se-enriched yeast in aquaculture feeds, resulting in enhanced I, Se, and Fe contents in fish muscle (Barbosa et al., 2020). Nevertheless, it is well acknowledged that the level of a nutrient in a portion of seafood does not allow to predict the amount of such nutrient that will be released from the food matrix and become available for absorption across the human intestinal epithelium during the digestion process, i.e., the bioaccessible fraction (Alves et al., 2018; Cardoso et al., 2015; Versantvoort et al., 2005). Bioaccessibility analysis plays an important role in risk-benefit assessment of the consumption of specific food or diets, since nutrients uptake depends not only on the ingested amount of a specific food item, but also on nutrients bioaccessibility (Cardoso et al., 2015; Girard et al., 2018; Hu et al., 2019). Several *in vitro* models have been developed to simulate the human digestion process, with static methodologies sequentially simulating oral, gastric, and intestinal phases with digestive juices (including the relevant enzymes in all steps) being the most used and reliable models to evaluate nutrients bioaccessibility in seafood (Alves et al., 2018; Cardoso et al., 2015; Torres-Escribano et al., 2011). Also, *in vitro* models are a cost-effective alternative to *in vivo* methods, since are less expensive, rapid, energy saving and allow controlling the experimental conditions for better reproducibility (Cardoso et al., 2015; He et al., 2010). This has led to an international effort to standardize them (Brodkorb et al., 2019; Minekus et al., 2014). Previous studies have used *in vitro* digestion methodologies to evaluate nutrients bioaccessibility in seafood and showed that oral bioaccessibility varies with the biochemical composition of the food matrix, processing, or preparation (Alves et al., 2018; Costa et al., 2016; Guérin et al., 2011; Lei et al., 2013; Moreda-Piñeiro et al., 2011, 2012). For instance, Alves and co-authors (2018) found that the cooking process reduces the bioaccessibility of toxic elements in seafood (i.e., MeHg and Cd), while a wider variability was found for essential elements (i.e., increased in fish for Zn, decreased in mussel for Fe and unchanged in fish and shellfish for Se and I). Despite attention has been recently given to trace elements bioaccessibility, including selenium and its species in seafood (Cabañero et al., 2007; Moreda-Piñeiro et al., 2011, 2013), to our knowledge, the existing information on iodine bioaccessibility in fish is still scarce (Ferraris et al., 2021). I and Se are essential nutrients for neurological and thyroid development, and seafood is a prominent dietary source (Bevis, 2015; FAO, 2020). Therefore, integrating bioaccessibility in the evaluation of the supply of these nutrients via seafood is of importance. Indeed, the few available studies addressing Se and I bioaccessibility in fortified foodstuff focused on vegetables (Hu et al., 2019; Nascimento da Silva et al., 2017; Pedrero et al., 2006).

In this context, the aim of the present study was to: 1) investigate the bioaccessibility of macro and trace elements (potassium, iodine, iron, selenium, and zinc), in gilthead seabream and common carp biofortified with I-rich seaweed (*L. digitata*) and Se-enriched yeast as feed ingredients with respect to non-biofortified counterparts; 2) evaluate the effect of steam-cooking on the bioaccessibility of macro and trace elements; and 3) assess the nutrients intake provided by the consumption of these fish items in relation to the relevant dietary reference values (DRVs). Seabream (*S. aurata*) and common carp (*C. carpio*) were selected as models since they are two of the most relevant and consumed farmed fish species (10% and 5% of European aquaculture production, respectively; 7% and 6% of European total apparent consumption, respectively) in Mediterranean countries and in central Europe, respectively (EUMOFA, 2021).

2. Material and methods

2.1. Growth trials and sampling

For each species, the feeding trial comprised two diets: a control feed (CTR), consisting in a commercial formulation covering the nutritional requirements for adult gilthead seabream and common carp, and an experimental biofortified feed (BF), supplemented with I-rich seaweed (*L. digitata*) and Se-enriched yeast blends (supplementary table S1). Gilthead seabream BF feed contained moderate levels of fishmeal (10%) and fish oil (3.8%) and was supplemented with a blend of microalgae (*Chlorella sp.*, *Tetraselmis sp.*, *Schizochytrium sp.*), macroalgae (*L. digitata*) and selenized yeast. Similarly, common carp BF feed, was formulated replacing half of the fishmeal with a blend of microalgae (*Spirulina* sp., *Chlorella* sp.), macroalgae (*L. digitata*) and selenized yeast. Additionally, vegetable oil was replaced by salmon oil extracted from by-products of farmed Atlantic salmon. Experimental extruded feeds were manufactured by SPAROS, Lda (Olhão, Portugal) and the enriched feeds 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, 2005, 2006).

The trial with gilthead seabream was conducted at SKALOMA farm facilities (Greece), whereas the common carp trial was conducted at the Fisheries Research Station of West Pomeranian University of Technology in Szczecin (Poland). Both trials were performed in compliance with the European guidelines on protection of animals used for scientific purposes (European Commission, 2007). Gilthead seabream specimens with an average initial body weight of 424 ± 21 g were distributed into a set of three cages (n = 490 fish per cage) placed in a coastal fish farm (39° 40' 16.77" N 20° 04' 22.70" E) and subjected to natural photoperiod (from August till November) with water temperature average of 23.4 ± 2.4 °C and a mean salinity of 36‰. The experimental feeding trial was tested in triplicate for 90 days (simulating a finishing diet). On the other hand, the common carp study was carried in a total of 6 cuboid cages of 3 m^3 placed in an earthen pond (53° 42' 5.99'' N 15° 21' 22.19'' E). Each cage was stocked with 100 fish (average initial body weight of 250 ± 10 g), and the feeding trial was conducted in triplicate for 116 days. Fish were hand-fed with equal portions, according to standard practices at the fish farms, and no mortality was observed during both gilthead seabream and common carp trials. For each species, final samplings were done 24 h after the last meal and 24 fish per treatment (CTR and BF) were sacrificed by immersion in chilled seawater (seabream) or freshwater (carp) following the commercial procedures employed in fish farms. At the end of the trial, all fish were measured, weighted (Table 1), and skinless fish muscle were collected (n = 3 pools of 8 fish each per treatment). For both gilthead seabream and common carp, one fish fillet (per specimen) was used for culinary steam-cooking, whereas the other fillet was used 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 were stored at– 80 °C until further analysis.

2.2. Steam-cooking procedure and proximate chemical composition

For each treatment and species, fish muscle samples were steam-cooked following the procedure previously described by Barbosa et al. (2021). Briefly, fish muscle samples were individually wrapped up in aluminum foil, steamed in an oven (Combi-Master CM 6, Rational

Table 1
Gilthead seabream (*S. aurata*) and common carp (*C. carpio*) biometric information before and after the feeding trial and fish muscle moisture (%) content before and after the culinary treatment.

	n	Total weight (g)	Total lenght (cm)	Muscle fillet			
				Moisture Raw (%)	Moisture Steamed (%)	Weight loss (%)	CY (%)
Gilthead seabream							
Baseline	9	413 ± 33	30 ± 1	72 ± 1	n.d.	n.d.	n.d.
CTR	24	598 ± 76	32. ± 1	71 ± 1	71 ± 1	8.1 ± 1.1	92 ± 4
BF	24	572 ± 74	32 ± 1	71 ± 1	70 ± 1	8.1 ± 1.6	92 ± 4
Common carp							
Baseline	9	261 ± 51	25 ± 2	81 ± 1	n.d.	n.d.	n.d.
CTR	24	1295 ± 90	37 ± 4	75 ± 1	71 ± 2	10 ± 1	92 ± 8
BF	24	1359 ± 37	37 ± 3	76 ± 1	73 ± 1	9 ± 2	92 ± 4

n, number of specimens analysed; n.d., not determined; CY, cooking yield; CTR, control diet; BF, biofortified diet. n.d. – not determined

GroßkÜcken Technik GmbH, Germany) at 105 °C for 15 min, and then cooled at room temperature. The final weight was registered to obtain the relevant cooking yield (CY), as the percentage ratio between cooked and raw fish muscle weight (Table 1), and the true retention (TR, %) for each element was calculated using following the equation (Eq. 1) (USDA, 2007):

$$TR = (\text{mean content of the element in cooked seafood/mean content of the element in raw seafood}) \times CY, \quad (1)$$

where CY = cooking yield.

2.3. In vitro human digestion procedure

Each raw and steamed fish sample was digested in duplicate using the same *in vitro* digestion protocol described by Alves et al. (2018). Briefly, 1.5 g of each fish homogenized sample were digested in Nalgene™ high-speed PPCO centrifuge tubes at 37 ºC using a Rotary Tube Mixer with Disc (25 rpm; LSCI, Portugal) in an incubator (Genlab, UK). The digestion steps were performed as follow: i) oral phase, where 4 mL of saliva fluid was added to the fish sample and incubated for 5 min at pH 7.0 ± 0.2; ii) gastric phase, where 8 mL of gastric fluid was added to the oral phase and incubated for 2 h at pH 2.0 ± 0.2); and iii) intestinal phase, where 8 mL of duodenal fluid and 4 mL of bile fluid were added to the gastric phase and incubated for 2 h at pH 7.0 ± 0.2 (digestion fluids composition are described in supplementary table S2). Enzyme degradation/inhibition was prevented by preparing each digestion fluid immediately before starting the digestion protocol, and the pH was adjusted immediately before each digestion step with NaOH (1 M) or HCl (1 M). At the end of the digestion, the process was stopped by placing the reaction tubes on ice, followed by centrifugation at 2750 *g* for 10 min at 10 ºC to separate the bioaccessible fraction (i.e., supernatant; BIO) from the sample pellet (non-bioaccessible fraction - NBIO). Negative controls containing the digestion fluids without fish sample were also performed. BIO and NBIO fractions were kept at– 80 ºC until analysis.

2.4. Analytical determination

2.4.1. Moisture content

Moisture content was determined in raw and steamed samples 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.

2.4.2. Essential elements

Essential elements were quantified in biofortified (BF) and non-biofortified (CTR) fish muscle samples (raw and steamed) before (BD) and after the *in vitro* digestion procedure. Each element in the bioaccessible (BIO) fraction (%) was calculated using the following ratio (Eq. 2):

$$\text{Bioaccessibility } (\%) = (\text{BIO} \times 100) / \text{BD} \quad (2)$$

where BIO corresponds to the element levels detected in the bioaccessible fraction and BD corresponds to the element levels detected in the sample before digestion.

2.4.2.1. Iodine (I) and selenium (Se). I and Se were determined by inductively coupled plasma mass spectrometry (ICP-MS). Raw and steamed fish muscle samples (BD) were homogenized, and 1 g was placed in high-pressure Teflon containers with 3 mL of HNO_3 67–69% v/v (ultrapure grade, Carlo Erba, Rodano, Italy) and 1 mL of H_2O_2 30% v/v (ultrapure grade, Sigma-Aldrich, Darmstadt, Germany). Samples were digested in a microwave system (UltraWAVE Single Reaction Chamber Microwave Digestion System, Milestone, Bergamo, Italy) with the following program: a) 23 mins to reach 240 °C; b) 10 mins at 240 °C (maximum power 1400 W); and c) 30 min depressurization and cooling to reach room temperature. I was determined by quadrupole ICP-MS using a Nexion 350D ICP-MS (Perkin Elmer, Waltham, MA, U.S.A.) equipped with a quartz concentric nebulizer and a cyclonic spray chamber (Waltham, MA, U.S.A.), whereas for Se a triple quadrupole ICP-MS/MS (Agilent 8800, Agilent Technologies Inc., Tokyo, Japan) equipped with a PFA (perfluoroalkoxy) concentric nebulizer and a double-pass PFA spray chamber cooled to 2 °C was used. The latter instrument was operated in MS/MS reaction mode by using oxygen as a reaction gas in mass shift; the analytical masses (SeO^+) were $m/z = 94$ and 96, which provided interference-free conditions. The quantitative determinations were carried out by the standard addition method. For iodine, an instrument tuning was performed daily prior to analysis to get the highest sensitivity at ^{127}I. Standards and samples were prepared in 1.5% (v/v) ammonia (Sigma Aldrich, Darmstadt, Germany) and 1% (v/v) isopropanol (Sigma Aldrich, Darmstadt, Germany), and quantitative determinations were carried out by external calibration. The bioaccessible fraction were diluted with the same mixture and analyzed with the standard addition method. To prevent memory effects in I determination, tetramethylammonium hydroxide (TraceSelect, Sigma Aldrich, Darmstadt, Germany) 0.5% (v/v) was used for rinsing the sample introduction system. For both I and Se, the standard solutions were obtained by diluting stock solutions (1 g L^{-1}, High-Purity, Charleston, SC, USA) with high-purity deionized water obtained from a Milli-Q Element system (Millipore, Molsheim, France).

2.4.2.2. Potassium (K), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn) and bromide (Br). K, Ca, Br, Cu, Fe and Zn were determined in raw and steamed fish muscle samples (BD) by energy dispersive X-ray fluorescence spectrometry (EDXRF). Briefly, freeze-dried muscle samples were ground for 2 min under 10 tons to make a cylindrical pellet with a diameter of 20 mm and a thickness of 1 mm. high-energy 3-D optics XRF spectrometer (Epsilon 5, PANalytical, Netherlands). It is equipped with a

600 W Sc/W-target X-ray tube with a beam spot diameter of about 18 mm. Between the X-ray tube and the specimen, a set of secondary targets is inserted in XYZ polarization geometry offering, mainly, the monochromatization of the exciting beam. In this study CaF_2, Ge and Mo secondary targets were selected. A Germanium detector with a nominal resolution of 140 eV for Mn-Kα was used for recording the X-ray fluorescence spectra and the acquisition time of each spectrum was adjusted for each secondary target and the operating conditions (Manousakas et al., 2018). For K and Fe, bioaccessible fractions (BIO) were determined using an ICP-OES (Thermo iCAP 6000 series), with radial and axial configuration. ICP-OES instrumental operating conditions were the following: Auxiliar Flow: 0.5 L min^{-1}, Plasma Orientation: radial or axial, RF power: 1200 W, Peristaltic pump's speed (Flush pump rate and analysis pump rate): 50 rpm, Pump stabilization time: 5 s, Integration time in UV and Visible: 15 and 10 s. For Cu, Zn and Br, bioaccessible fractions (BIO) were diluted in Milli-Q water, filtered through 0.45 μm filters before ICP-MS analyses (ThermoX Series II, Thermo Fisher Scientific, Bremen, Germany). ICP-MS operating conditions were optimized daily, and standard solutions from single elements high purity ICP stock standards (Inorganic Ventures and SCP Science) were used.

2.4.3. Quality control

All reagents used in the analyses were of high analytical grade and water was ultra-purified (18.2 MΩ cm) using a Milli-Q-Integral system (Merck, Germany). Analytical quality was assessed through reference materials, including fish muscle (ERM®-BB422) from the European Commission – Joint Research Centre Institute for Reference Materials and Measurements (IRMM) (Geel, Belgium), dogfish muscle (DORM-2) from the National Research Council of Canada (Ontario, Canada) and oyster tissue (SRM 1566b) from the National Institute of Standards and Technology (Gaithersburg, USA). The obtained values agreed with certified values (supplementary table S3). Limits of detection (LODs) and quantification (LOQs) are presented in supplementary table S3. The LOD was assigned to the detection limit (DL) of the calibration curve (DL = 3 × standard deviation (σ) of response at the zero-concentration level) and the LOQ was calculated as (3 × LOD).

2.5. Nutritional contribution (NC)

The NC of BF and CTR fish muscle consumption was calculated according to reference values set for individual adults (> 18 years old), pregnant women and children (1–3 years) by the European Food Safety Authority and the following equation (Eq. 3):

$$NC\ (\%) = 100 \times (C \times M) / DRV \quad (3)$$

where C = concentration of the element in μg g^{-1}; M = typical meal portion in g (150 g for adults and pregnant women and 75 g for children); DRV = adequate intake (AI) for I, Se, Cu and K (EFSA, 2014a,b, 2015b,c, 2016), or population reference intake (PRI) for Ca, Fe and Zn (EFSA, 2014c, 2015d). Whenever the NC was greater than 100%, the tolerable upper intake level (UL), i.e., the maximum levels of total chronic daily intake (from all sources) which is not expected to pose a risk of adverse health effects to humans (EFSA, 2022) was also considered.

2.6. Statistical analysis

Data were analyzed for normality of distribution and homoscedasticity using Kolmogorov–Smirnov and Levene's tests, respectively, and data were Log-transformed, whenever necessary, to comply with the assumptions of normality (Kolmogorov–Smirnov's test) and homogeneity of variances (Levene's test). The effect of diet (BF and CTR) and culinary treatment (raw or steamed) on fish fillets elements content was tested by factorial analysis of variance (ANOVA). Post-hoc Tukey HSD test was applied in group multiple comparisons to identify significant differences. Statistical significance was set at $P < 0.05$. Analyses were carried out using STATISTICA™ (Version 7.0, StatSoft Inc., Tulsa, Oklahoma, USA).

3. Results

3.1. Trace and macro elements in biofortified farmed fish

BF gilthead seabream fillets presented significantly higher contents of I and Se compared to CTR fillets (Fig. 1, supplementary table S4). Additionally, steaming significantly increased Cu, I, Se, and Zn contents only in BF fillets, although Cu levels were lower compared to CTR fillets. Higher TR values of Cu, I, Se, and Zn were found in BF fillets (>100%).

Concerning common carp, BF fillets presented statistically higher contents of Br, Cu, I, Se, and Zn compared to CTR (Fig. 2, supplementary table S4). For Cu, this higher content was found only in raw BF fillets compared to CTR. Steaming significantly increased I content in CTR fillets, as well as Se content in BF fillets with TRs values above 100%. In contrast, steaming significantly decreased Cu content in BF fillets, where the lowest TR value (66%) was observed.

3.2. Bioaccessibility of trace and macro elements in biofortified farmed fish

In both BF and CTR gilthead seabream fillets, Fe, I, K, Se, and Zn bioaccessibility was higher than 60% regardless of the culinary treatment (Fig. 3, supplementary table S5). The most bioaccessible elements were I and Zn, and Fe in raw fillets only. Yet, significantly lower K bioaccessibility was only observed in raw BF fillets compared to CTR. Steaming significantly decreased I (BF) and Fe (CTR and BF) bioaccessibility.

Regardless of the culinary treatment, for common carp fillets, I bioaccessibility was lower than 50% (CTR and BF), while Se and K bioaccessibility varied between 50% (CTR) and 70% (BF), and Fe and Zn bioaccessibility was higher than 70% (Fig. 4, supplementary table S5). BF fillets presented significantly higher bioaccessibility of I compared to CTR (< LOQ), as well as of Se (in steamed fillets).

Bioaccessible Br, Ca and Cu values for both gilthead seabream (CTR and BF) and common carp (CTR and BF) fillets could not be determined as the results were below the limit of quantification (supplementary table S5).

3.3. Nutritional contribution of consumption of biofortified farmed fish

The consumption of a 150 g (adults and pregnant women) or 75 g (children) portion of raw and steamed BF gilthead seabream fillets contributed to higher intakes in relation to DRVs for I (up to 10% for children and up to 12% for adults) and Se (up to 44% for pregnant women and > 100% for children), compared to CTR fillets (Table 2). Additionally, steaming significantly increased the Se NC in BF fillets for all population groups, as well as the Cu NC in both CTR and BF fillets. Despite exceeding the daily adequate intake, both CTR and BF fillets contributed Se within the UL for children (up to 24% for raw and up to 27% for steamed). In terms of elements bioaccessibilty, BF fillets (raw and steamed) contributed to higher intakes in relation to DRVs for I (up to 6% for pregnant women and up to 9% for adults) and Se (up to 28% for pregnant women and up to 80% for children).

Concerning common carp, the consumption of a 150 g (adults and pregnant women) or 75 g (children) portion of raw and steamed BF fillets contributed to higher intakes in relation to DRVs for I (up to 8% for pregnant women and up to 11% for adults) and Se (> 100% for all population groups), compared to CTR fillets (Table 2). Despite exceeding the daily adequate intake, both raw and steamed BF carp fillets contributed Se within the UL for all population groups (up to 47% for adults/pregnant women and up to 85% for children). Steaming significantly increased Se NC in BF fillets for all population groups.

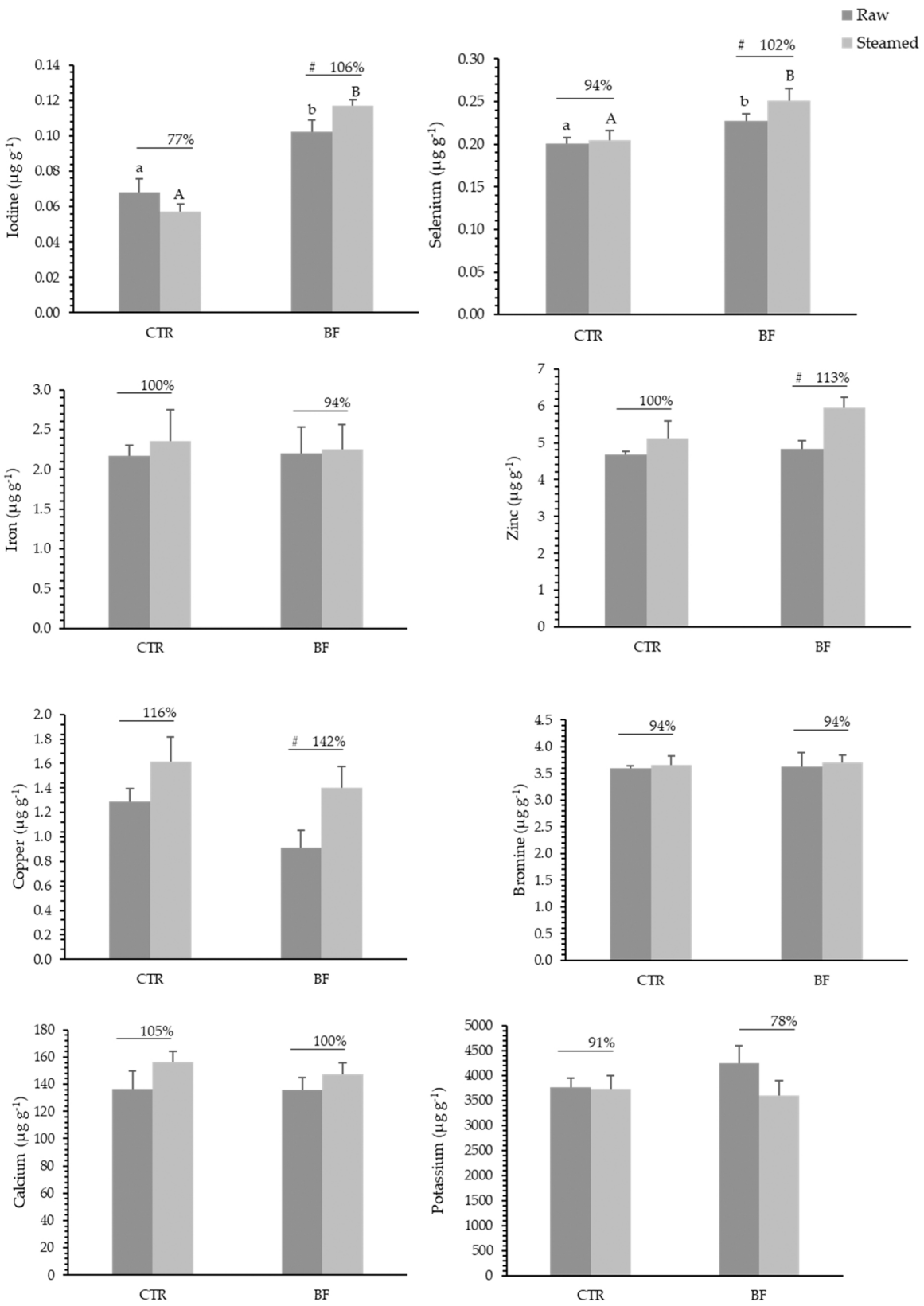

Fig. 1. Levels of trace (iodine, selenium, iron, zinc, copper, bromine) and macro (calcium, potassium) elements in biofortified (BF) and non-biofortified (CTR) gilthead seabream fillets (average ± SD, in wet weight) prior to *in vitro* digestion, and element true retention (TR) values in fish fillet after steaming. Different lowercase and upper-case letters indicate significant differences ($P < 0.05$) between CTR and BF fish fillets, in raw (grey) and steamed (light grey) samples, respectively. For each treatment (CTR and BF), # represents significant differences ($P < 0.05$) between raw and steamed fillets.

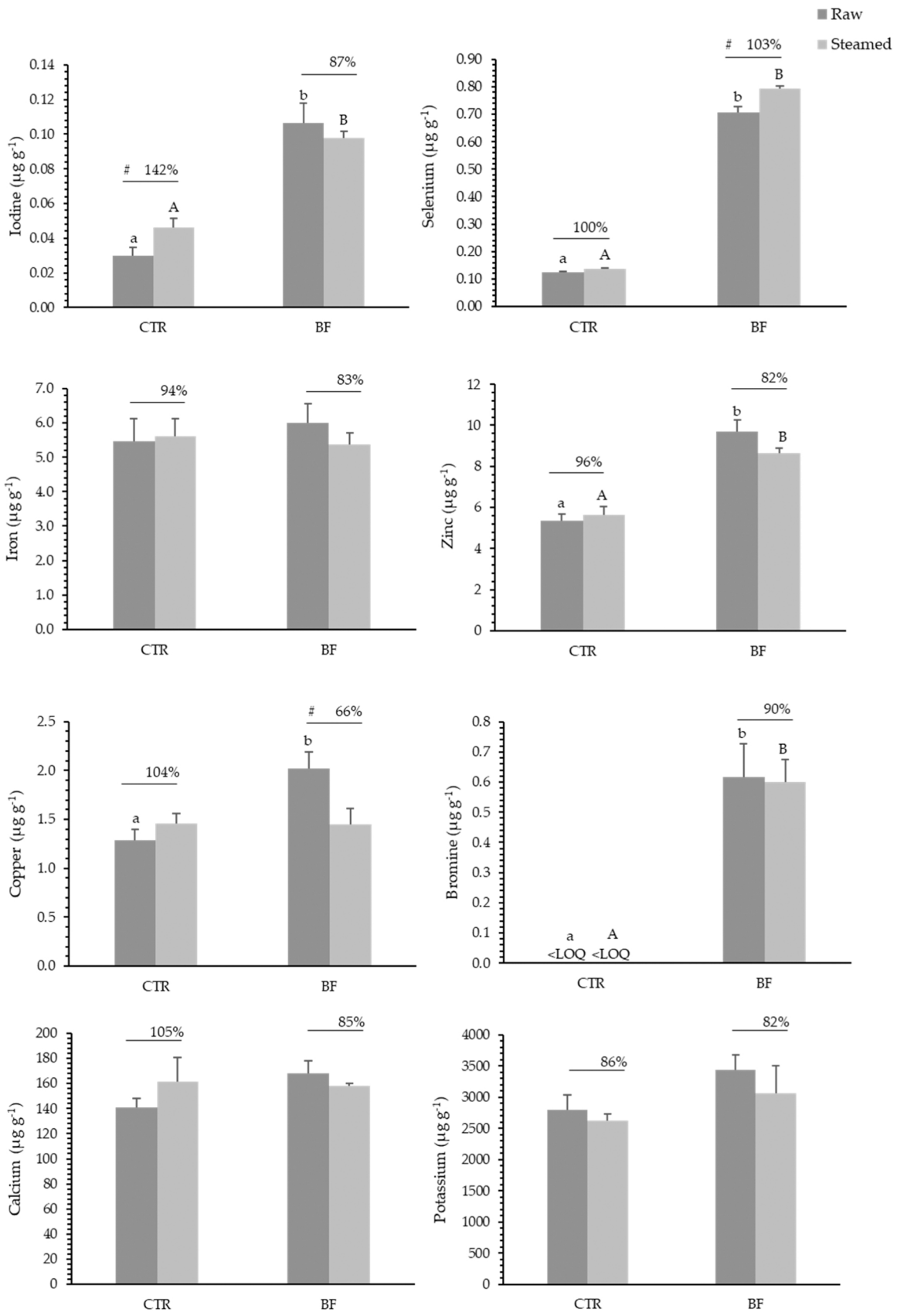

Fig. 2. Levels of trace (iodine, selenium, iron, zinc, copper, bromine) and macro (calcium, potassium) elements in biofortified (BF) and non-biofortified (CTR) common carp fillets (average ± SD, in wet weight) prior to *in vitro* digestion, and element true retention (TR) values in fish fillet after steaming. Different lower-case and upper-case letters indicate significant differences ($P < 0.05$) between CTR and BF fish fillets, in raw (grey) and steamed (light grey) samples, respectively. For each treatment (CTR and BF), # represents significant differences ($P < 0.05$) between raw and steamed fillets.

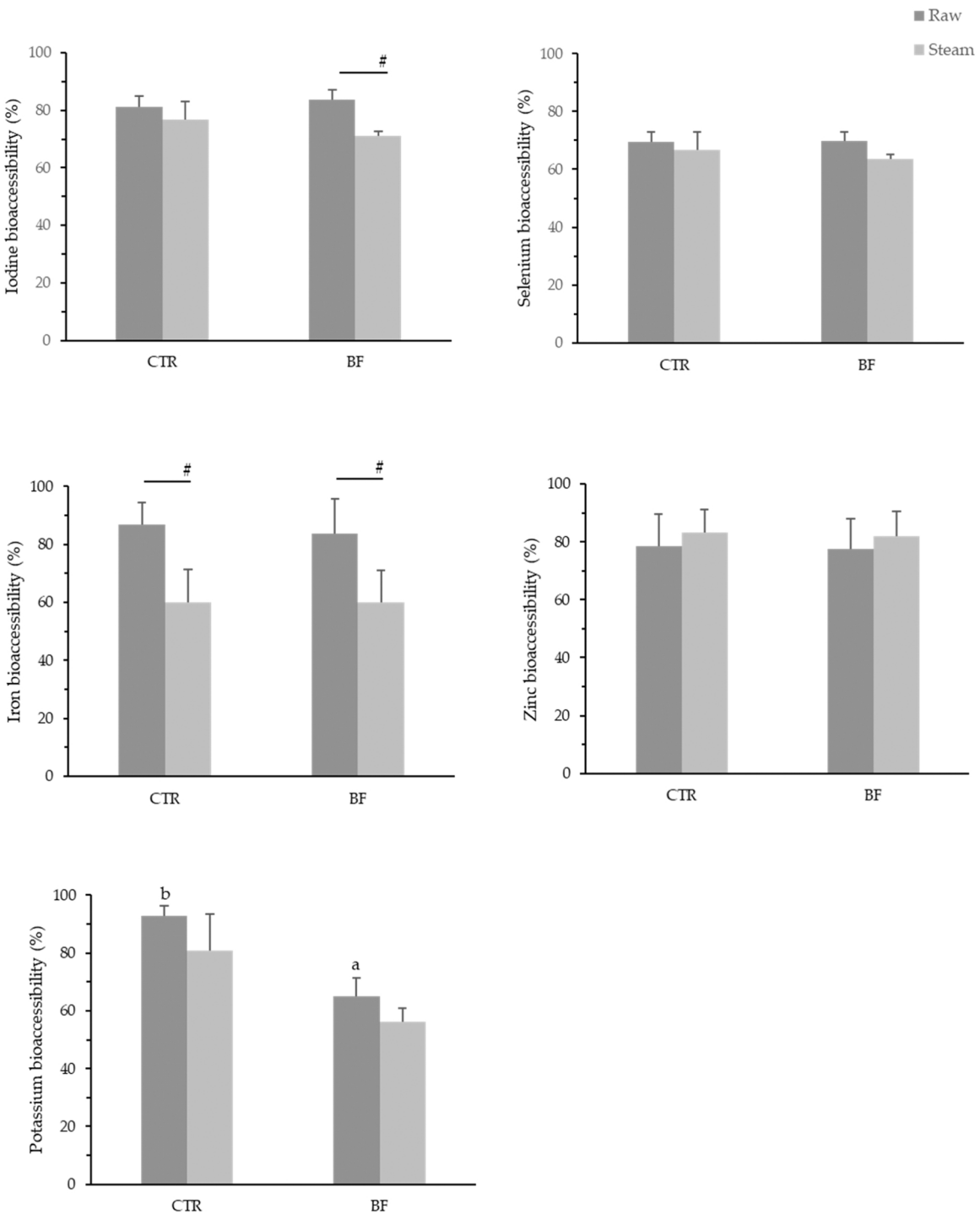

Fig. 3. Bioaccessibility (%) of trace (iodine, selenium, iron, zinc), and macro (potassium) elements in biofortified (BF) and non-biofortified (CTR) gilthead seabream fillets (average ± SD). Different lower-case and upper-case letters indicate significant differences ($P < 0.05$) between CTR and BF fish fillets, in raw and steamed samples, respectively. For each treatment (CTR and BF), $^{\#}$ represents significant differences ($P < 0.05$) between raw and steamed fillets.

Additionally, the consumption of raw BF fillets contributed to higher intakes in relation to the AI of Cu for all population groups (from 18% for adults to 22% for children and premenopausal women) and steaming significantly reduced them (14% for adults and 16% for children and premenopausal women). Higher NCs of Zn were also found for the consumption of raw and steamed BF fillets (up to 13% for pregnant women and adults and up to 17% for children). Taking into account the elements bioaccessibility, BF fillets (raw and steamed) increased the NCs of I (up to 3% for pregnant women and up to 5% for adults) and Se (up to 92% for pregnant women and >100% but within 56% of the UL for children).

4. Discussion

4.1. Effects of the biofortification strategy on elements content in farmed fish fillets

In line with previous studies undertaken at pilot scale (Barbosa et al.,

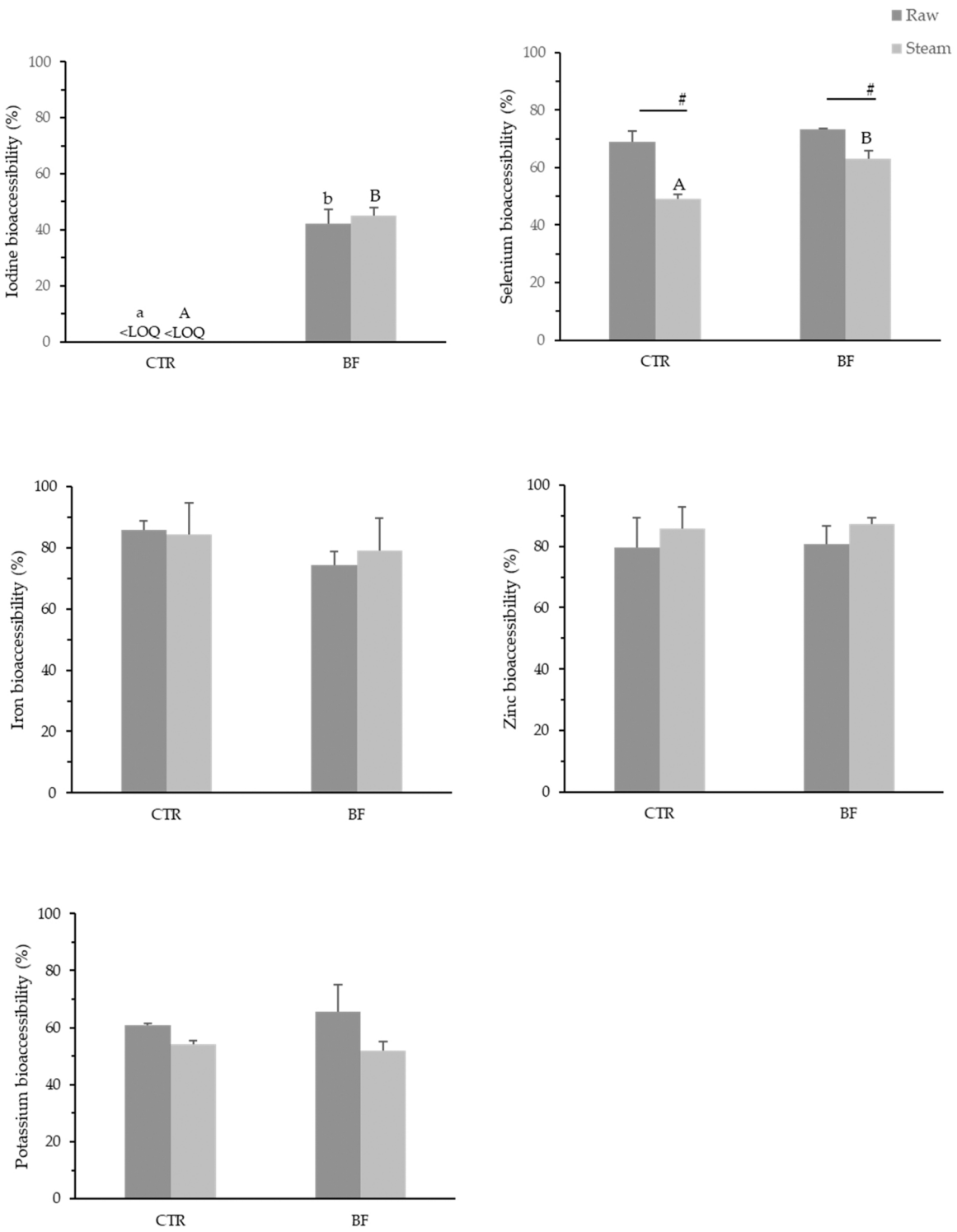

Fig. 4. Bioaccessibility (%) of trace (iodine, selenium, iron, zinc), and macro (potassium) elements in biofortified (BF) and non-biofortified (CTR) common carp fillets (average ± SD). Different lower-case and upper-case letters indicate significant differences ($P < 0.05$) between CTR and BF fish fillets, in raw and steamed samples, respectively. For each treatment (CTR and BF), $^{\#}$ represents significant differences ($P < 0.05$) between raw and steamed fillets.

2020, 2021), the incorporation of iodine-rich seaweed (*L. digitata*) and Se-rich yeast in gilthead seabream and common carp feeds resulted in enhanced content of I and Se, but also of other essential elements, in fish fillets. Similar findings for I were observed in previous studies focused on marine species, namely gilthead seabream (*S. aurata*), and freshwater species, namely rainbow trout (*Oncorhynchus mykiss*) and char (*Salvelinus sp.*), biofortified with I-rich seaweed supplemented diets (Ramalho Ribeiro et al., 2015, 2017; Schmid et al., 2003; Valente et al., 2015). Successful Se biofortification was also reported in rainbow trout (*O. mykiss*) using a similar dietary approach (Ramalho Ribeiro et al., 2017). The present biofortification strategy (incorporation of approximately 0.5% of *L. digitata* and 0.03% Se-yeast as part of the diet) was more effective in common carp (3.6-fold increase in I and 5.7-fold increase in Se) than in gilthead seabream (1.5-fold increase in I and 1.1-fold increase in Se) in comparison with non-biofortified fish (CTR). This certainly depends on the lower I and Se baseline levels in fillets of

Table 2
Nutritional contribution (%) of non-biofortified (CTR) and biofortified (BF) 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 the consumption of a portion of 75 g of fish for children.

		Gilthead seabream								Common carp							
		CTR[1]		BF[1]		CTR[2]		BF[2]		CTR[1]		BF[1]		CTR[2]		BF[2]	
		raw	steamed	raw	steamed	raw	steamed	raw	steamed	raw	steamed	raw	steamed	raw	steamed	raw	steamed
Trace elements																	
Cu	Adults, males[4]	11 ± 1	15 ± 2$^{\#}$	8.5 ± 1.4	13 ± 2$^{\#}$	n.d.	n.d.	n.d.	n.d.	12 ± 1^{a}	14 ± 1	18 ± 2^{b}	14 ± 2$^{\#}$	n.d.	n.d.	n.d.	n.d.
	Adults, females[4]	14 ± 1	19 ± 2$^{\#}$	11 ± 2	16 ± 2$^{\#}$	n.d.	n.d.	n.d.	n.d.	15 ± 1^{a}	18 ± 1	22 ± 2^{b}	16 ± 2$^{\#}$	n.d.	n.d.	n.d.	n.d.
	Pregnant women[5]	12 ± 1	16 ± 2$^{\#}$	9.1 ± 1.4	14 ± 2$^{\#}$	n.d.	n.d.	n.d.	n.d.	13 ± 1^{a}	15 ± 1	19 ± 1^{b}	15 ± 2$^{\#}$	n.d.	n.d.	n.d.	n.d.
	Children[6]	13 ± 1	17 ± 2$^{\#}$	9.8 ± 1.6	15 ± 2$^{\#}$	n.d.	n.d.	n.d.	n.d.	14 ± 1^{a}	16 ± 1	22 ± 2^{b}	16 ± 2$^{\#}$	n.d.	n.d.	n.d.	n.d.
Fe	Adults[4]	3.1 ± 0.2	3.2 ± 0.5	3.1 ± 0.5	3.1 ± 0.4	2.5 ± 0.2	2.1 ± 0.2	2.7 ± 0.3	2.1 ± 0.1	7.5 ± 0.9	7.7 ± 0.7	8.2 ± 0.8	7.3 ± 0.5	6.7 ± 0.8	6.1 ± 0.3	5.7 ± 0.2	5.1 ± 0.7
	Premenopausal women[4]	2.0 ± 0.1	2.2 ± 0.4	2.1 ± 0.3	2.1 ± 0.2	1.7 ± 0.1	1.4 ± 0.2	1.9 ± 0.2	1.4 ± 0.1	5.1 ± 0.5	5.3 ± 0.5	5.6 ± 0.5	5.0 ± 0.3	4.6 ± 0.6	4.2 ± 0.2	3.9 ± 0.1	3.5 ± 0.5
	Children[6]	2.3 ± 0.1	2.5 ± 0.4	2.4 ± 0.4	2.4 ± 0.3	2.1 ± 0.1	1.6 ± 0.2	2.1 ± 0.1	1.6 ± 0.1	5.9 ± 0.7	6.1 ± 0.6	6.4 ± 0.6	5.8 ± 0.4	5.3 ± 0.6	4.8 ± 0.2	4.5 ± 0.1	4.0 ± 0.5
I	Adults[4]	6.8 ± 0.7^{a}	5.7 ± 0.4^{A}	10 ± 1^{b}	12 ± 0^{B}	5.6 ± 0.8^{a}	4.4 ± 0.4^{A}	8.6 ± 0.8^{b}	8.3 ± 0.1^{B}	3.1 ± 0.5^{a}	4.6 ± 05^{A}	11 ± 1^{b}	9.8 ± 0.4^{B}	n.d.^{a}	n.d.^{A}	4.5 ± 0.8^{b}	4.3 ± 0.1^{B}
	Pregnant women[5]	5.1 ± 0.6^{a}	4.3 ± 0.3^{A}	7.7 ± 0.5^{b}	8.8 ± 0.2^{B}	4.2 ± 0.6^{a}	3.3 ± 0.3^{A}	6.4 ± 0.7^{b}	6.2 ± 0.1^{B}	2.2 ± 0.4^{a}	3.4 ± 0.4^{A}	8.0 ± 0.9^{b}	7.3 ± 0.3^{B}	n.d.^{a}	n.d.^{A}	3.4 ± 0.8^{b}	3.2 ± 0.1^{B}
	Children[6]	5.7 ± 0.6^{a}	4.8 ± 0.3^{A}	4.8 ± 0.3^{b}	9.8 ± 0.3^{B}	4.7 ± 0.6^{a}	3.7 ± 0.3^{A}	7.1 ± 0.8^{b}	6.9 ± 0.1^{B}	2.5 ± 0.4^{a}	3.8 ± 0.4^{A}	8.9 ± 0.9^{b}	8.2 ± 0.3^{B}	n.d.^{a}	n.d.^{A}	3.8 ± 0.9^{b}	3.5 ± 0.1^{B}
Se	Adults[4]	43 ± 1^{a}	44 ± 2^{A}	49 ± 2^{b}	54 ± 3$^{B\#}$	30 ± 1^{a}	29 ± 0^{A}	34 ± 2^{b}	34 ± 2^{B}	27 ± 1^{a}	29 ± 1^{A}	> AI (42 ± 1)^{b}	> AI (47 ± 1)$^{B\#}$	18 ± 1^{a}	14 ± 1^{A}	> AI (31 ± 1)^{b}	> AI (30 ± 1)^{B}
	Pregnant women[5]	35 ± 1^{a}	36 ± 2^{A}	40 ± 2^{b}	44 ± 3$^{B\#}$	25 ± 1^{a}	24 ± 0^{A}	28 ± 2^{b}	28 ± 2^{B}	22 ± 1^{a}	24 ± 1^{A}	> AI (42 ± 1)^{b}	> AI (47 ± 1)$^{B\#}$	15 ± 1^{a}	12 ± 1^{A}	92 ± 3^{b}	91 ± 1^{B}
	Children[6]	> AI (22 ± 1)^{a}	> AI (22 ± 1)^{A}	> AI (24 ± 1)^{b}	> AI (27 ± 2)$^{B\#}$	70 ± 2^{a}	68 ± 1^{A}	79 ± 5^{b}	80 ± 5^{B}	63 ± 2^{a}	69 ± 2^{A}	> AI (76 ± 2)^{b}	> AI (85 ± 1)$^{B\#}$	43 ± 3^{a}	34 ± 1^{A}	> AI (56 ± 2)^{b}	> AI (55 ± 1)^{B}
Zn	Adults, males[4]	6.0 ± 0.1	6.6 ± 0.6	6.2 ± 0.3	7.6 ± 0.4	4.7 ± 0.1	5.7 ± 0.5	5.0 ± 0.1	6.2 ± 0.4	6.9 ± 0.4^{a}	7.2 ± 0.5^{A}	13 ± 1^{b}	11 ± 1^{B}	5.5 ± 0.4^{a}	6.0 ± 0.5^{A}	10 ± 1^{b}	9.3 ± 0.1^{B}
	Adults, females[4]	7.5 ± 0.2	8.2 ± 0.8	7.8 ± 0.4	9.6 ± 0.5	6.0 ± 0.1	7.1 ± 0.6	6.2 ± 0.2	7.7 ± 0.5	8.7 ± 0.5^{a}	9.1 ± 0.6^{A}	16 ± 1^{b}	14 ± 1^{B}	6.9 ± 0.4^{a}	7.6 ± 0.6^{A}	13 ± 1^{b}	12 ± 1^{B}
	Pregnant women[5]	6.4 ± 0.1	7.0 ± 0.7	6.7 ± 0.3	8.2 ± 0.4	5.1 ± 0.1	6.1 ± 0.5	5.3 ± 0.1	6.6 ± 0.4	7.4 ± 0.4^{a}	7.8 ± 0.5^{A}	13 ± 1^{b}	12 ± 1^{B}	5.9 ± 0.5^{a}	6.5 ± 0.5^{A}	11 ± 1^{b}	10 ± 1^{B}
	Children[6]	8.2 ± 0.2	8.9 ± 0.8	8.4 ± 0.4	10 ± 0.5	6.4 ± 0.1	7.7 ± 0.7	6.7 ± 0.2	8.4 ± 0.5	9.4 ± 0.5^{a}	9.8 ± 0.7^{A}	17 ± 1^{b}	15 ± 1^{B}	7.5 ± 0.6^{a}	8.2 ± 0.6^{A}	14 ± 1^{b}	13 ± 1^{B}
Macro elements																	
Ca	Adults[4]/Pregnant women[5]	2.2 ± 0.2	2.5 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	n.d.	n.d.	n.d.	n.d.	1.5 ± 1.3	1.7 ± 1.5	2.7 ± 0.2	1.7 ± 1.4	n.d.	n.d.	n.d.	n.d.
	Children[6]	2.3 ± 0.2	2.6 ± 0.1	2.3 ± 0.1	2.5 ± 0.1	n.d.	n.d.	n.d.	n.d.	1.6 ± 1.4	1.8 ± 1.6	2.8 ± 0.2	1.8 ± 1.5	n.d.	n.d.	n.d.	n.d.
K	Adults[4]	16 ± 1	16 ± 1	18 ± 2	15 ± 1	15 ± 1	13 ± 1	12 ± 1	8.8 ± 0.9	12 ± 1^{a}	11 ± 1	15 ± 1^{b}	13 ± 2	7.1 ± 0.21	5.9 ± 0.1	10 ± 1	7.4 ± 0.1
	Pregnant women[5]	14 ± 1	14 ± 1	16 ± 1	13 ± 1	13 ± 1	11 ± 1	11 ± 1	7.7 ± 0.8	11 ± 1^{a}	10 ± 1	13 ± 1^{b}	12 ± 1	6.1 ± 0.2	5.2 ± 0.1	10 ± 1	6.5 ± 0.1
	Children[6]	35 ± 2	35 ± 3	40 ± 3	34 ± 3	33 ± 1	28 ± 2	27 ± 1	19 ± 2	26 ± 2^{a}	25 ± 1	32 ± 2^{b}	29 ± 4	16 ± 2	15 ± 2	18 ± 3	13 ± 2

[1]Values calculated without considering element bioaccessibility; [2]Values calculated considering element bioaccessibility. Values are mean ± standard deviation. The Nutritional contribution (NC; %) are presented for [4]adults (> 18 years) with mean body weight in Europe (70 kg), [5]pregnant/lactating women with mean body weights in Europe (67 kg) and [6]children (1–3 years) with mean body weight in Europe (13 kg) set by EFSA (2012). The percentages of NC were calculated accordingly the Dietary Reference Values (DRVs), namely Adequate Intakes (AI) or population reference intake (PRI), as well as the tolerable upper intake level (UL; in parenthesis) set by EFSA (2014a, 2014b, 2014c 2015a,2015c, 2015d, 2016, 2019, 2022). For Zn, was selected the PRI for LPI of 600 (EFSA, 2014c). Different superscript small letters represent statistical differences ($p < 0.05$) between CTR and BF fish fillets, in raw and steamed samples, respectively. # represents significant differences ($P < 0.05$) between raw and steamed fillets. n.d. – not determined.

conventional common carp, but other factors might also be involved. It is known that I and Se biofortification effectiveness depends on the fish and seaweed species (i.e., origin and size), and Se-rich yeast used, as well as the timing and duration of feeding (Barbosa et al., 2020; Ramalho Ribeiro et al., 2017). Indeed, the effectiveness of biofortification with I and Se using the same approach (incorporation of *L. digitata* and Se-yeast as part of the diet) was previously reported to vary in the same fish species (Barbosa et al., 2020). One point is the substrate used for biofortification: the incorporation of higher percentages of *L. digitata* (0.8%) as part of the diet, resulted in a lower enhancement of I content in gilthead seabream fillets (1.4-fold increase), which is explained by the initial I concentration in the seaweed specimens used resulting in different I levels in feeds (13.3 ± 0.2 mg kg^{-1} versus 20.4 ± 0.6 mg kg^{-1}). Moreover, the incorporation of lower percentages of Se-rich yeast (0.01%) as part of the diet, resulted in lower enhancement of Se content in common carp fillets (1.4-fold increase). In terms of content of other elements than the biofortified ones, the inclusion of microalgae blends with different mineral compositions and absolute concentrations, especially *Spirulina sp.* (Pereira et al., 2019), resulted in higher contents of Br, Ca, Cu, K and Zn in BF common carp fillets, in contrast to gilthead seabream. In line with previous studies (Barbosa et al., 2021; Ramalho Ribeiro et al., 2015), the results of this study demonstrate that steaming significantly increases I content, but only in BF gilthead seabream fillets and in CTR common carp fillets. On the other hand, Alves and co-authors (2018) reported that steaming did not affect I content in hake (*Merlucius australis*), monkfish (*Lophius piscatorius*), mackerel (*Scomber scombrus*), tuna (*Katsuonus pelamis*), and plaice (*Pleuronectes platessa*). An increase in Se content was also previously reported in steamed BF gilthead seabream (Barbosa et al., 2021), in boiled, grilled, and roasted gilthead seabream (Afonso et al., 2018), and in blue shark (*Prionace glauca*) after grilling and steaming (Matos et al., 2015); though, this was likely associated with water loss during culinary treatment (Alves et al., 2018; Erkan, 2011; Martins et al., 2011). Higher retention of I and Se (TR $\geq$ 80%) after streaming are mainly associated to higher cooking yields (CY $>$ 90%) and the fact that these elements are mainly bound to proteins, i.e., are less prone to leaching during steam-cooking (Barbosa et al., 2021; Oliveira et al., 2019; Vicente-Zurdo et al., 2019). Overall, elements' true retentions after steaming were high in the present study, indicating that this culinary procedure has no detrimental effect in elements content in BF fish fillets from both species. In contrast with the authors' previous study, BF gilthead seabream presented a higher cooking yield (CY of 92% versus 84%), resulting in higher elements retention after steaming, and ultimately less minerals leaching from muscle (Bastías et al., 2017). Indeed, no significant changes were observed in gilthead seabream fillets moisture composition after steaming, indicating that steam cooking has less influence in fillets elemental composition. In contrast, despite BF common carp fillets also presented higher CY compared to the earlier study (92% versus 80%), lower retention of Cu, Fe, and Zn was observed, reflecting losses during steam-cooking. In fact, decreased moisture content was observed in common carp fillets after steaming, which may explain some minerals leaching due to water loss, evaporation, and/or dehydration (Oliveira et al., 2019; Sobral et al., 2018). The present results demonstrate that the biofortification strategy enhanced the farmed fish nutritional quality and steaming is a healthy cooking method, preserving health-valuable nutrients. In addition, it is confirmed that the overall fish elemental composition is closely related with the origin, size, and initial elemental content of the biofortification substrate, as reported earlier (Barbosa et al., 2020, 2021; He et al., 2010; Mnari et al., 2012; Petricorena, 2015).

4.2. I, Se, Fe, K, and Zn bioaccessibility in biofortified farmed fish

Fish supplies the human diet with several essential elements and is a good source of some of them, especially I, Se, and to a certain extent Fe (partially present in haem form), having vital roles in human health (EFSA, 2014d; Cilla et al., 2019; Gharibzahedi & Jafari, 2017). In this sense, developing and designing biofortification strategies considering economical and sustainable solutions is a cost-effective measure to supply essential nutrients to the global population (FAO et al., 2021). Still, nutrients absorption from biofortified food is overall limited, especially in seafood. Bioaccessibility is the major determinant to be investigated in this respect since a nutrient has to be first released from the food matrix by the digestive process to become available for absorption in the human intestine. In general, the bioaccessibility of essential elements in BF and CTR gilthead seabream and common carp were above 60%, except I bioaccessibility in common carp (less than 50%). Previous studies also reported overall good bioaccessibility of I and Se with some variability depending on the fish species and culinary treatment. For example, bioaccessibility of I reached 98% in blue whiting (*Micromesistius poutassou*) (Ferraris et al., 2021), whereas it was only 47% in tuna (*K. pelamis*) (Alves et al., 2018). Similarly, bioaccessibility of Se was found to vary in a range from 59% in tuna (*K. pelamis*) to 76% in swordfish (*Aphanopus carbo*), 83% in sardine (*Sardina pilchardus*) (Cabañero et al., 2004), 87% in monkfish (*L. piscatorius*) (Alves et al., 2018), 90% in gilthead seabream (*S.aurata*) (Afonso et al., 2018) and in blue shark (*P. glauca*) (Matos et al., 2015). The high bioaccessibility of I and Se may be explained by the strong association of these elements with soluble proteins that are easily broken down by digestive enzymes (Afonso et al., 2018). Noteworthy, the differences observed in different species are likely to be related with fish proximate chemical composition, since protein and fat content may affect I and Se solubility, as well as enzymes efficiency (Cabañero et al., 2004; Doh et al., 2019). To what extent this effect observed in the *in vitro* digestion procedure is relevant *in vivo* (i.e., in humans) needs to be ascertained.

Steaming induced a decrease in I bioaccessibility only in BF gilthead seabream fillets, but not in CTR gilthead seabream and common carp. Similarly, in another study steaming did not affect I bioaccessibility in tuna (*K. pelamis*) (Alves et al., 2018). In the present study, steaming decreased Se bioaccessibility in common carp fillets (BF and CTR), but not in gilthead seabream. Decreased Se bioaccessibility was also reported after steaming in blue shark (*P. glauca*) (Matos et al., 2015) and plaice (*P. platessa*) (Alves et al., 2018), whereas increased Se bioaccessibility was reported in steamed mackerel (*S. scombrus*) (Alves et al., 2018), and in boiled, grilled, and roasted gilthead seabream (*S. aurata*) (Afonso et al., 2018). Overall, cooking procedures may lead to a decrease in elements bioaccessibility due to the leaching of unbound elements or of elements in protein complexes as a result of protein denaturation (Amiard et al., 2008). Muscle myofibrils denaturation and contraction may result in insoluble protein-elements complexes, leading to less digestible and bioavailable nutrients in the muscle tissues (Amiard et al., 2008; Doh et al., 2019; He et al., 2010).

Concerning other elements, high bioaccessibility of Fe (up to 80% in seabream and up to 70% in carp) and Zn (up to 70% in seabream and up to 80% in carp) were observed compared to previous studies. In fact, Fe bioaccessibility in previous studies was 69% in tuna (*K. pelamis*) (Alves et al., 2018), up to 58% in seabass (*Lateolabrax japonicus*) and 52% in red seabream (*Pagrosomus major*) (He et al., 2010). On the other hand, in previous studies Zn bioaccessibility was up to 70% in seabass (*L. japonicus*) and up to 67% in red seabream (*P. major*), and 71% in hake (*M. australis*), 40% in plaice (*P. platessa*) and 28% in tuna (*K. pelamis)* (Alves et al., 2018). In these earlier studies Fe bioaccessibility significantly decreased in steamed red seabream (*P. major*) (He et al., 2010) and Zn bioaccessibility increased in steamed hake (*M. australis*), in plaice (*P. platessa*) and in tuna (*K. pelamis*) (Alves et al., 2018). As far as K is concerned, a high bioaccessibility (80%) was reported in raw and cooked tilapia (*O. niloticus*) (Santos et al., 2022). Still, the bioaccesibility of K was relatively high in the present study, reaching 65% in gilthead seabream and 66% in common carp. The high bioaccesibility of K may be explained by the fact that this element occurs in food matrix as simple ions, being easily solubilized into the gastrointestinal tract (Santos et al.,

2022). For Cu, bioaccessibility was reported to range in seabass (*L. japonicus*) and red seabream (*P. major*) between 70% and 85% (He et al., 2010). Lower Cu bioaccessibility (40%) was reported in tuna (*K. pelamis*) (Alves et al., 2018). Limited Cu bioaccessibility may be related with the storage of this element in the form of less easily digestible proteins and less easily degraded complexes formed by metallothioneins and insoluble ligands, especially after cooking (Amiard et al., 2008).

In general, elemental bioaccessibility depends not only on the food matrix, but especially on changes in the chemical structure, mobility, and solubility of nutrients (Doh et al., 2019; Liu et al., 2017). In terms of the steam-cooking effect on elemental bioaccessibility, the present results indicate a species-specific effect in accordance with previous findings (He & Wang, 2013). The different biofortification approaches for gilthead seabream and common carp contributed to distinct effects on fish elemental composition and the steam-cooking treatment does not seem to remarkably affect fillets elemental composition (see TRs values). In general, BF fillets elemental bioaccessibility was above 65%, except for I in common carp. Steam-cooking affected differentially the elemental bioaccessibility, and showed that, excluding I in common carp, K was the least digestible element in steamed BF fish fillets from both species.

4.3. Nutritional benefits of biofortified farmed fish consumption to human health

Considering the consumption of a portion of 150 g for adults/pregnant women and 75 g for children, steamed BF gilthead seabream and common carp significantly improved the NC of I and Se compared to CTR fish. These results are consistent with the authors' previous findings (Barbosa et al., 2021). In comparison with the previous study, BF gilthead seabream fillets presented a similar NC for I (12% for adults, 9% for pregnant women and 10% for children) and lower for Se, which nevertheless exceeded the AI (i.e. >100%) and amounted to 54%, 44% and 27% of the UL for adults, pregnant women and children, respectively. It is worth mentioning that in the present study, *L. digitata* and Se-rich yeast were supplemented to gilthead seabream diets at lower levels (i.e., 0.5% against 0.8% and 0.03% against 0.04%, respectively). On the other hand, BF common carp fillets showed a lower NC for I (10% for adults, 7% for pregnant women and 8% for children), but a much higher NC for Se (more than 100% for all population groups). In this case, *L. digitata* was supplemented to fish diets at the same levels (0.54%), while Se-rich yeast was supplemented at higher levels (0.03% against 0.01%). Furthermore, in line with our previous study, increased NC of Zn was observed in BF common carp fillets compared to CTR. Nevertheless, lower NC of Fe (up to − 15%), Cu (up to − 8%) and Zn (up to − 15%) were achieved, compared to previous results (Barbosa et al., 2021). When it comes to elements bioaccessibility, a reduction was observed in all elements NC in both gilthead seabream and common carp, compared to elements NC before the *in vitro* digestion process, being consistent with previous findings in hake (*M. australis*), tuna (*K. pelamis*), monkfish (*L. piscatorius*), mackerel (*S. scombrus*), and plaice (*P. platessa*) (Alves et al., 2018). Despite the observed reduction in NC based on elements bioaccessibility, BF fish fillets from both species still presented an enhanced nutritional value, providing increased intakes of I and Se, two important nutrients, in relation to the respective DRVs. Furthermore, the consumption of BF gilthead seabream and common carp fillets seems to be a good alternative to improve consumers' I intakes avoiding I overexposure, compared to seaweed (*L. digitata*) consumption, that may increase consumers risk of exceeding the UL set for I (Alves et al., 2018). Considering the present results, higher NC for I and Se are reached through the consumption of BF gilthead seabream and higher NC for I, Se and Zn are achieved through the consumption of BF common carp. Therefore, the present biofortification approach with I-rich seaweed (*L. digitata*) and Se-rich yeast in gilthead seabream and common carp may contribute to reduce I and Se suboptimal intakes in target population groups, whereas for common carp a fine-tuning of Se biofortification, with lower supplemented levels, seems advisable to avoid exceedance of the UL upon regular consumption, taking into account the Se intake from the rest of the diet.

5. Conclusions

The dietary strategies devised through the supplementation with I-rich seaweed and Se-rich yeast were highly efficient in both gilthead seabream and common carp produced at commercial scale. Biofortified gilthead seabream and common carp fillets revealed enhanced I and Se contents, which were impacted by steam cooking to a limited extent. Additionally, the bioaccessibility of I, Se, Fe and Zn turned out to be above 70% in BF gilthead seabream and common carp fillets, with the exception of I in common carp (42%).

In general, BF gilthead seabream and common carp fillets were found to be effective in improving the nutritional contribution of I and Se, even though a fine-tuning of Se biofortification in common carp, with lower supplemented levels, seems advisable to avoid exceedance of the UL upon regular consumption. Overall, this study revealed that biofortification strategies are viable solutions to reduce deficiencies of essential elements in human populations, especially concerning I and Se, with no detrimental impact on most of the other essential elements. Further studies on different biofortification strategies (i.e., supplementation by other natural ingredients from sustainable sources) are warranted to provide insights in order to develop eco-innovative and cost-effective farmed fish with nutritional benefits to human health.

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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.

Data Availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105760.

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