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In vitro bioaccessibility of essential minerals from raw and cooked Tilapia fillet: Method validation and analysis by synchronous vertical dual view ICP OES

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ABSTRACT

A novel insight into the nutritional changes of *Tilapia* fillets due to heat treatments was provided by evaluating the influence of baking and air-frying on the total mass fraction and bioaccessibility of micronutrients Ca, Cu, Fe, K, Mg, P, S and Zn. A static *in vitro* model was used to simulate the human gastrointestinal digestion and then quantify the analytes using inductively coupled plasma optical emission spectrometry with a synchronous vertical dual view system (SVDV ICP OES). Unlike recent studies focusing on the mineral content of cooked foods, we interpreted the results more accurately by considering the weight yield factors. Compared with the raw sample, the baking and air-frying procedures significantly increased the total mass fraction of Fe. Air-frying promoted a slight decrease in Zn content. Bioaccessibility results ranged from 12–31% to 86–88% for Zn and K, respectively. Both heat treatments appear to increase the bioaccessibility of Mg, Fe, and Zn, whereas the other evaluated micronutrients were not remarkably affected by cooking. In addition, the SVDV mode was an efficient instrumental strategy for determination of total, bioaccessible, and non-bioaccessible fractions of essential minerals in *Tilapia*, improving the sample throughput.

1. Introduction

Fishes and fisheries products are a significant part of the animal protein consumed by the population. With annual *per capita* consumption substantially increasing (FAO. Food & Agriculture Organization of the United Nations, 2020), fish provide high-quality proteins, essential amino acids, vitamins, and, especially, ω−3 class of polyunsaturated fatty acids. Fish consumption has been associated with several health benefits, including potential action against neurodegenerative- and cardiovascular-related diseases and hepatoprotection properties (Chen et al., 2022; FAO. Food & Agriculture Organization of the United Nations, 2020). Moreover, fish species are good sources of micronutrients such as essential minerals in human nutrition.

Essential minerals play a fundamental role in maintaining the proper functioning of the human body. In this sense, minerals such as calcium and phosphorus are vital for healthy bones and teeth. Our body needs potassium and magnesium for suitable fluid balance and transmission of nerve impulses, respectively. In addition, copper and zinc are essential in many enzymatic reactions and immune systems. Iron is required to produce hemoglobin, acting mainly as oxygen-carrying to the body cells (Gharibzahedi & Jafari, 2017; Zoroddu et al., 2019). As the body itself cannot synthesize these micronutrients, they must be ingested from food such as fish and fisheries products.

Among the freshwater fish species, *Tilapia* (*Oreochromis niloticus*) is the most widespread in Brazil, accounting for approximately 60% of Brazilian fish farming. The latest data point to significant growth of *Tilapia* production in the country, reaching more than 486,000 tons in 2020 (Peixe, 2021). The consumption of *Tilapia* as fillets form is quite common, and it has already been shown that this product constitutes a potential source of essential minerals in the human diet (Farzad et al., 2019; Islam et al., 2021). However, to define the nutritional value, it is necessary to know the amounts available for intestinal absorption through bioaccessibility studies.

Bioaccessibility is defined as the fraction of a nutrient released from the food matrix into the gastrointestinal (GI) tract after the food digestion process, becoming potentially available for further absorption by the intestinal cells (Cardoso et al., 2015). Bioaccessibility studies can be carried out *in vivo*, considered the gold standard trial, or using *in vitro* models, able to simulate the physicochemical and physiological conditions of the human GI tract (Lucas-González et al., 2018), followed by analysis of the resulting extract, or chyme. To overcome the drawbacks related to *in vivo* experiments, *e.g.*, high cost, time demanding, analytical and ethical restrictions, static *in vitro* digestion assays have been

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usually applied for estimating the bioaccessible fraction of minerals in several fisheries products by spectroanalytical methods (Afonso et al., 2018; Alves et al., 2018; Jiang et al., 2021; Schmidt et al., 2021).

Raw *Tilapia*-based dishes are enjoyed (*e.g.* sushi). Still, it is mainly consumed after cooking methods such as boiling, baking, or frying/air-frying. In this context, the effects of thermal processing on the nutritional value of *Tilapia* have been investigated. Recent studies showed that boiling, roasting, and frying had a significant influence on the lipid, taste/nutrition (several metabolites), and aroma (volatile organic compounds) profile in *Tilapia* fillets (Chen et al., 2021; Li et al., 2021, 2022; Shi et al., 2019). Moreover, Zhang et al. (2020) reported that boiling and frying decreased the *in vitro* bioaccessibility of polybrominated diphenyl ethers in the muscle tissue of *Tilapia*. However, to the best of our knowledge, studies addressing the influence of heat treatments on the total mass fraction and bioaccessibility of essential minerals in *Tilapia* fillets have not been published.

Different dry-cooking methods, *e.g.*, grilling, baking, or frying are responsible for changes in mineral content. The increases in essential minerals from cooked samples are generally associated with weight loss (as water and/or fat) (Afonso et al., 2018; Alves et al., 2018; Kalogeropoulos et al., 2012). However, the expression or interpretation of these results usually does not consider weight yield factors. The yield factors (YF) describe the changes in the food weight after cooking methods and are useful to adjust the losses or increase of water and fat during the process (FAO. Food And Agriculture Organization of The United Nations, 2016). Similar approaches have been applied to express the results in studies of dializability of essential minerals and toxic elements, avoiding underestimated or overestimated values in cooked seafood analysis (Schmidt et al., 2021, 2018).

Considering the large consumption of *Tilapia* and the knowledge gap related to the influence of heat treatments on its mineral content, we develop a precise and accurate method of multi-elemental analysis to evaluate the influence of baking and air-frying on the total mass fraction and *in vitro* bioaccessibility of Ca, Cu, Fe, K, Mg, P, S, and Zn in *Tilapia* fillets by SVDV-ICP OES. We must also remark that information about heat treatments on the bioaccessibility of nutrients is important to know the actual nutritional value of the food. In addition, we also estimated the nutritional contribution from the raw and cooked *Tilapia* for the recommended daily allowance of these micronutrients.

2. Material and methods

2.1. Instrumentation

A model 5110 inductively coupled plasma optical emission spectrometer (Agilent, Santa Clara, CA, USA) equipped with synchronous vertical dual view (SVDV) arrangement, and an OneNeb® series 2 nebulizer (Agilent, Santa Clara, CA, USA) was used for determination of total mass, bioaccessible and non-bioaccessible fractions of Ca, Cu, Fe, K, Mg, P, S, and Zn. The instrumental conditions applied to ICP OES are shown in Table S1 (see Supplementary Material). High purity argon (> 99.99%) from White Martins-Praxair (Sertaozinho, Brazil) was used for instrument gas supply. A microwave-assisted digestion system (Multiwave Go, Anton-Paar GmbH, Graz, Austria) equipped with 12 PTFE-TFM vessels and a 30 positions digester block (Tecnal, Piracicaba, Brazil) with 60 mL PFA vessels (Savillex, Eden Prairie, MN, USA) were used for samples preparation. For *in vitro* gastrointestinal digestion, a Dubnoff shaking thermostatic bath (Novatecnica, Sao Paulo, Brazil) was employed to incubate the samples. An analytical balance resolution to 0.0001 g (AUW-220, Shimadzu, Kyoto, Japan) was used to weigh the samples. A pH-meter (W3B, BEL, Monza, Italy) and a centrifuge (Excelsa II 206, FANEM, Sao Paulo, Brazil) were used for pH adjustment and supernatant-residue separation, respectively. A freeze-dryer (E-C MicroModulyo, Edwards, UK) and an analytical mill (IKA-Werke, Staufen, Germany), were used for samples drying and milling, respectively. An air-fryer pan (Saúde Inox, Philco, Manaus, Brazil) and an electric oven (Minioven Super Chef, Arno, São Paulo, Brazil) were used for the dry-cooking procedures.

2.2. Reagents and solutions

All solutions were prepared using ultrapure water (resistivity 18.2 MΩ cm) obtained from a Milli-Q purification system (Merck KGaA, Darmstadt, Germany). Nitric acid previously purified in a sub-boiling acid distillation apparatus (Berghof, Eningen, Germany) and hydrogen peroxide solution (30 wt.%) from Sigma-Aldrich (Saint Louis, MO, USA) were used for samples preparation procedures. The simulated digestive fluids for *in vitro* assay were prepared using the following reagents and enzymes: sodium bicarbonate ACS EMSURE® (Merck KGaA, Darmstadt, Germany), hydrochloric acid previously purified in a sub-boiling distillation apparatus, sodium hydroxide (reagent grade ≥ 98%), pepsin from porcine gastric mucosa P7000 (≥ 250 units/mg), pancreatin from porcine pancreas P1750 (4x USP specifications), and bile salts (≈ 50% sodium cholate and 50% sodium deoxycholate), all from Sigma-Aldrich (Saint Louis, MO, USA). Working measurement standards for Ca, Cu, Fe, K, Mg, P, S, and Zn determination were prepared from ICP grade 1000 mg L^{-1} single stock standard solutions from Specsol (Sao Paulo, Brazil). Stock solutions of Y, Ge and Bi (Specsol, Sao Paulo, Brazil) were used for internal standardization. All glassware/plasticware were decontaminated by soaking in a 10% (v v^{-1}) HNO_3 solution for at least 24 h and further rinsed with ultrapure water.

2.3. Samples and pre-treatments

Approximately 0.8 kg of *Tilapia* (*Oreochromis niloticus*) packaged into a plastic container was purchased as frozen fillets from a local market in Sao Carlos, Brazil, and stored in a freezer at –20 °C until use. After natural thawing at room temperature, *Tilapia* fillets were gently washed with ultrapure water and cut into twelve pieces with sizes of approximately 5 cm x 5 cm (length *x* width) using a ceramic knife. The resultant weight of each piece was 28 ± 2 g (wet weight). One part ($n = 8$ pieces) was separated to carry out the heat treatments, and the other ($n = 4$ pieces) was kept for raw sample analysis.

2.4. Heat treatments of Tilapia fillets

Based on the more traditional ways for *Tilapia* fillets consumption by Brazilian population, we selected two dry-heat treatments: (*1) Frying in air-fryer* - approximately 110 g of *Tilapia* fillets ($n = 4$ pieces) was weighed and placed in the pre-heated air-fryer pan and fried for 10 min at 180 °C; and (*2) Baking in electric oven* - approximately 110 g of *Tilapia* fillets ($n = 4$ pieces) was weighed, covered up in aluminum foil and placed to an aluminum baking dish and baked in the pre-heated household electric oven for 20 min at 180 °C. No seasoning or additional ingredients were used in both cooking procedures. After the heat treatments, *in natura* and cooked samples were weighed, freeze-dried and milled. The moisture contents were determined by evaluating the loss of weight after freeze-drying.

2.5. In vitro gastrointestinal digestion

To simulate the stomach and small intestine phases, we used an *in vitro* protocol based on static conditions for gastrointestinal digestion (Laparra et al., 2003). The gastric fluid was simulated by using a 10% m v^{-1} pepsin solution prepared in 0.1 mol L^{-1} HCl medium, whereas the intestinal fluid was simulated with a suspension composed of 0.2% m v^{-1} pancreatin and 1.25% m v^{-1} bile salts, prepared in a 0.1 mol L^{-1} $NaHCO_3$ solution. These fluids were prepared immediately before each experiment to ensure enzymatic activity. In addition, previous experiments for pH adjustment in each digestive stage were carried out.

Approximately 0.5 g of freeze-dried *Tilapia* fillets was weighed into a 50 mL-polypropylene flask, and 9.5 mL of ultrapure water was added.

After 15 min, the pH of the mixture was adjusted to 2.0 using a 6.0 mol L^{-1} HCl solution, and 200 μL of the simulated gastric fluid (SGF) was added. The flasks were sealed with Parafilm® and closed with the cap. Then, the samples were incubated into a horizontal-shaking thermostatic bath at 37.0 ± 0.5 °C and 150 rpm for 2 h to mimic the gastric digestion stage. After incubation, the flasks were placed in an ice bath for 15 min to stop the pepsin enzymatic activity. Subsequently, we added 2.5 mL of simulated intestinal fluid (SIF) to the gastric digest, and the pH of the food-fluid mixture was adjusted to 7.0 using a 1.0 mol L^{-1} NaOH solution. The samples were incubated for a second time (37 °C and 150 rpm) for 2 h to mimic the intestinal digestion stage. At the end of incubation, the flasks were placed in an ice bath to stop the enzymatic action of the pancreatin components. Finally, the samples were centrifugated at 3600 rpm for 20 min to separate the supernatant (gastrointestinal digest or chyme) from the residual fraction. A schematic representation of the *in vitro* protocol is shown in Fig. S1 (see Supplementary Material). *In vitro* gastrointestinal digestion assays were performed in triplicate. Analytical blanks were prepared by adding 9.5 mL of ultrapure water and the same amounts of simulated digestive fluids. The bioaccessibility of each essential mineral from *Tilapia* fillet was calculated according to Eq. (1).

$$\text{Bioaccessibility } (\%) = \frac{[\text{An}]_{\text{bio}}}{[\text{An}]_{\text{total}}} x\ 100 \quad (1)$$

where $[\text{An}]_{\text{bio}}$ is the bioaccessible fraction of the analyte expressed as mg kg^{-1} ww, and $[\text{An}]_{\text{total}}$ is the total mass fraction of the analyte, expressed as mg kg^{-1} ww.

2.6. Analytical procedures for samples preparation

In natura and cooked *Tilapia* fillets were microwave-assisted digested using diluted acid to determine the total mass fraction of minerals. For this purpose, 0.2 g of freeze-dried fillet was weighed in digestion vessels, and 3.0 mL of purified HNO_3 + 3.0 mL of H_2O + 2.0 mL of 30% wt H_2O_2 solution were added. Then, a 3-steps heating program was applied: (1) 20-min ramp to reach 180 °C; (2) holding at 180 °C by 20 min; and (3) automatic cool-down of the vessels. After digestion, the samples were diluted up to 30 mL. All experiments were performed in triplicate. Analytical blanks were prepared similarly, without the addition of samples into the digestion vessels.

To minimize eventual carbon matrix effects, the *in vitro* gastrointestinal digests (chyme) were also microwave-digested. An aliquot of 4.0 mL of the chyme was digested using 4.0 mL of a 7.0 mol L^{-1} HNO_3 plus 2.0 mL of 30% wt H_2O_2 solution. The same heating program previously described was applied. The resulting solution was diluted up to 20 mL. Analytical blanks were prepared using 4.0 mL of the blank chyme (see Section 2.5) and the same amount of digester solution.

The residual fraction was digested in a digester block equipped with PFA closed vessels. Entire the residue was transferred to reactional vessels, and 5.0 mL of concentrated HNO_3, 3.0 mL of ultrapure water, and 2.0 mL of 30% wt H_2O_2 solution were added. Then, the vessels were heated at 100 °C for 3.5 h. After digestion, we diluted the resulting solution up to 50 mL and filtered it using a 0.45 μm syringe filter to determine the residual (or non-bioaccessible) fraction. We prepared the analytical blanks using the entire blank residue and the same amount of digester solution.

2.7. Multi-element determination

The total mass, bioaccessible, and residual fractions of Ca, Cu, Fe, K, Mg, P, S, and Zn in raw and cooked *Tilapia* fillet were determined by synchronous vertical dual view (SVDV) ICP OES using the external calibration method. The SVDV arrangement is an instrumental improvement based on a device capable of combining emission signals from radial and axial views, allowing the simultaneous determination of macro and trace elements in the same measurement. This configuration provides higher sensitivity for less abundant minerals by analyzing them axially while using radial viewing for more abundant ones (Donati et al., 2017). In all cases, an appropriate dilution was used to the SVDV ICP OES instrument calibrated with acid-matched standard calibration solutions, and residual acidity and total dissolved solids (TDS) did not exceed 10% v v^{-1} and 1% m v^{-1}, respectively. The working standards were prepared from 1000 mg L^{-1} single stock elements with the measuring intervals ranging from 0 to 0.1 mg L^{-1} for Cu and Fe; 0–0.5 mg L^{-1} for Zn; 0–10 mg L^{-1} for Ca and Mg; and 0–100 mg L^{-1} for K, P, and S. The linear curves had correlation coefficients of at least 0.999, with a standard error < 3% (Table S7, Supplementary Material). The instrumental conditions for chyme and residue analysis were assessed using spike-recovery experiments. For this purpose, Y, Ge, and Bi were evaluated as internal standards to verify eventual transport effects on the ICP OES performance, and the results were compared with external calibration.

2.8. Quality control

The analytical procedures for total mass and bioaccessible fractions determination were validated through background equivalent concentration (BEC), detection limits (LOD), quantification limits (LOQ), measurement precision, and measurement trueness. In addition, we also assessed possible matrix effects on the quantification of bioaccessible fractions. The LOD and LOQ values were calculated from the signal-background ratio (SBR) and the background equivalent concentration (BEC) (LOD = 3 *x* BEC *x* RSD_{Br}/100, and LOQ = 10 *x* BEC *x* RSD_{Br}/100). Measurement precision was expressed as repeatability (n = 5) from the relative standard deviation (% RSD) after analysis of the raw sample. The measurement trueness of the method applied for the determination of total mass fractions was assessed by analysis of certified reference materials of Fish protein (NRCC DORM-4) and Dogfish liver (NRCC DOLT-5). The measurement trueness of the *in vitro* bioaccessibility method was evaluated through a mass balance study, *i.e.*, a comparison between the sum of bioaccessible plus the residual fraction and the total mass fraction of each analyte (Eq. (2)). Mass balance was performed for raw and cooked *Tilapia* fillets.

$$\%\ \text{MB} = \frac{[\text{An}]_{\text{bio}} + [\text{An}]_{\text{res}}}{[\text{An}]_{\text{total}}}\ x\ 100 \quad (2)$$

where $[\text{An}]_{\text{bio}}$ and $[\text{An}]_{\text{res}}$ are, respectively, the average bioaccessible and residual fractions of the analyte expressed as mg kg^{-1} (dry weight, dw); and $[\text{An}]_{\text{total}}$ is the total mass fraction of the analyte expressed as mg kg^{-1} dw.

2.9. Data treatment and statistical analysis

Except for the mass balance study, the total mass and bioaccessible fractions were expressed as wet weight (ww), considering the weight yield factors (*YF*) for the cooked samples (Eq. (3)). These factors were applied to adjust for eventual losses of water and fat during the baking and air-frying of *Tilapia* fillets.

$$[\text{An}]_{\text{ww}} = [\text{An}]_{\text{dw}} \cdot YF \cdot \frac{(100 - \%\ \text{moisture})}{100} \quad (3)$$

where $[\text{An}]_{\text{ww}}$ is the analyte mass fraction expressed as wet weight; $[\text{An}]_{\text{dw}}$ is the analyte mass fraction found in the freeze-dried sample (dry weight), and '% moisture' is the moisture content of the cooked sample. As no additional ingredient or cooking medium was added, *YF* was calculated by the ratio between the weight of the cooked and raw samples (Semedo Tavares et al., 2018).

Retention factors for each mineral in cooked *Tilapia* was calculated by using the true retention method (%TR), as shown in Eq. (4) (USDA. U.S. Department of Agriculture, 2007).

$$\%\text{TR} = \frac{[\text{Min}]_{\text{cooked}}}{[\text{Min}]_{\text{raw}}} \cdot \text{YF}.100 \quad (4)$$

where $[\text{Min}]_{\text{cooked}}$ and $[\text{Min}]_{\text{raw}}$ is the mineral content (wet-weight) in cooked and raw sample, respectively.

The values and their respective uncertainties measurement were expressed as average ± standard deviation ($n = 3$). To evaluate whether the differences between the total mass and bioaccessible fraction of the minerals from raw and cooked *Tilapia* fillets differ significantly, an one-way analysis of variance (ANOVA) followed by the Tukey's HSD test for multiple means comparison was applied.

3. Results and discussion

3.1. Moisture content and weight yield factors (YF)

After the heat treatments, the moisture content decreased from 75% in raw *Tilapia* fillets to 71 and 64% for baked and air-fried, respectively. The intense heat from air-flowing around the food, enhancing the heat penetration into the *Tilapia* fillets, could explain the higher water loss after air-frying (Zaghi et al., 2019). Weight losses (as water and fat) by 17 and 40% were observed after baking and air-frying, respectively. Based on these results, the calculated *YF* values were 0.83 and 0.60 for baking and air-frying heat treatment, respectively. These results are in accordance with the values described in the fish database for dry-cooked fish samples (FAO. Food And Agriculture Organization of The United Nations, 2016).

3.2. Validation of the analytical method for total mass fraction determination

Diluted acid for sample preparation has become a trend to replace the conventional procedures based on concentrated reagents, meeting some Green Chemistry requirements. According to a previous study (Higuera et al., 2021), we used a 2-fold diluted HNO_3 plus H_2O_2 for raw and cooked *Tilapia* sample preparation to determine the total mass fraction of Ca, Cu, Fe, K, Mg, P, S, and Zn by SVDV ICP OES. Table S2 (see Supplementary Material) presents the figures of merit of the analytical procedure. The LOD and LOQ were obtained considering the calculated *YF* values, sample mass, and dilution factors used in the sample preparation. The LOQs ranged from 0.07 to 29 mg kg^{-1} for Zn and K, respectively, considered suitable to allow the mineral determination of all analyzed samples, except for Cu in raw *Tilapia* fillet. RSDs were less than 6% for all evaluated minerals, indicating an adequate measurement precision (repeatability) for the analytical procedure.

The measurement trueness was evaluated by analyzing certified reference materials of Fish protein and Dogfish liver ($n = 3$). As presented in Table S3 (see Supplementary Material), the elemental mass fractions found in the CRMs agreed with the reference quantity values, with recoveries in the 85 – 115% range. Besides improving the sample throughput compared to the conventional dual-view system, the use of SVDV mode provided satisfactory analytical performance. These results indicate that the method was suitable for elemental determination in fish samples.

3.3. Influence of heat treatments on the total mass fraction of essential minerals

After evaluating the analytical method, we apply it to determine Ca, Cu, Fe, K, Mg, P, S, and Zn in raw and cooked *Tilapia* fillet to estimate possible changes on the total mass fraction of the analytes after dry-heat treatments. The results were expressed as wet weight considering the weight yield factors for the cooked samples (see Eq. (3)). Figs. 1 and 2 present the total mass fraction of the essential minerals.

The highest total mass fractions were found for potassium, ranging from 4221 (baked sample) to 4347 mg kg^{-1} (raw sample). These values were higher than those found in raw *Tilapia* fillets purchased from the US marketplace (3030 mg kg^{-1}) (Farzad et al., 2019). No significant differences were observed for potassium mass fraction in cooked samples or between the raw and air-fried *Tilapia*. Despite the statistical difference shown by Tukey's test ($p < 0.05$), the baking did not cause a remarkable effect on the potassium mass fraction, as the loss was only 3% when compared with raw *Tilapia* fillet. As described for other fish species such as *Trout, meager,* and *Seabream* (Afonso et al., 2018; Costa et al., 2013; Gokoglu et al., 2004), high retention of potassium also was obtained after *Tilapia* cooking.

Raw and cooked samples presented similar phosphorus and sulfur contents (≈ 2000–2500 mg kg^{-1}). The total mass fraction of P ranged from 1942 to 2005 mg kg^{-1}, showing no statistical difference between the three treatments ($p > 0.05$). No influence of air-frying on the sulfur mass fraction was observed (average of 2321 mg kg^{-1} for raw and air-fried cooking procedure), in contrast to the slight increase of 8% obtained after baking (average content of 2497 mg kg^{-1}). Other authors identified similar trends for *meager* and *Seabass* after grilling, roasting, and baking procedures (Badiani et al., 2013; Costa et al., 2013).

The heat treatments did not cause a significant effect on the total mass fraction of calcium. In raw and cooked samples, Ca average content was approximately 100 mg kg^{-1}, similar to the value previously obtained by Farzad et al. (2019) in raw *Tilapia* fillet. For Mg, the total mass fraction found in cooked samples was close to 280 mg kg^{-1}, accounting for a slight decrease of 5% compared to the raw sample (293 mg kg^{-1} ww). Other authors also observed no influence of grilling and roasting on the Ca content in *Seabream* but reported a significant increase of Mg after both cooking methods (Afonso et al., 2018).

The total mass fraction of cooper was below the LOQ (< 0.13 mg kg^{-1}) in the raw sample. The average content for air-fried and baked *Tilapia* fillets was 0.19 and 0.23 mg kg^{-1}, respectively, and no statistical differences ($p > 0.05$) were observed. A significant decrease of Cu (≈ 38%) and Zn (≈ 63%) were obtained in flesh *Tilapia* after baking at 300 °C, probably due to the release of these minerals with soluble amino acids and uncoagulated proteins caused by the high cooking temperature (Atta et al., 1997). Schmidt and co-authors did not observe significant differences in Cu content in seafood after the cooking procedure (Schmidt et al., 2021). Otherwise, the heat treatments significantly increased the Fe mass fraction ($p < 0.05$), ranging from 1.7 mg kg^{-1} in raw *Tilapia* fillet to 2.3 mg kg^{-1} after cooking procedure. Kalogeropoulos et al. (2012) reported an increased Fe content in several fish species after pan-frying and grilling methods, attributing this effect to the water loss during cooking. On the other hand, the Fe total content significantly decreased (≈ 50%) in *Salmon* fillets after oven-baking at 250 °C (Bastías et al., 2017).

Raw and cooked samples presented zinc mass fractions from 3.3 to 4.0 mg kg^{-1}, similar to the found in tissue muscle of *in natura* red *Tilapia* (Low et al., 2015). Compared to raw *Tilapia* fillets, we observe a decrease of 8% in the Zn mass fraction in the samples prepared in the air-fryer and an increase of 8% in baked *Tilapia*. Possible leaching from the aluminum recipient and consequent rise of Zn in grilled *Seabass* fish was reported in the literature (Lomolino, Crapisi & Cagnin, 2016). However, the increase of Zn mass fraction after *Tilapia* baking (with aluminum foil) in the present study was not as remarkable as that observed in *Seabass*. Thus, we can not conclude that the aluminum foil or dish affected the Zn content.

In addition, it is important to highlight that the aluminum foil used in baking also does not increase the Al content of *Tilapia* fillets. For raw and baked samples, the total mass fraction of Al (data not shown) was below the LOQs (< 1.5–1.8 mg kg^{-1}). These results were in accordance with those previously found in the literature. No significant effects were observed for Al content in seafood after roasting, grilling, and pan-broiling using aluminum foil at different cooking conditions (Mol & Ulusoy, 2020).

3.3.1. Mineral retention factors for cooked Tilapia

The retention factors can be used to estimate the nutrient proportion remaining in the cooked food in comparison to the content originally present in raw food (USDA. U.S. Department of Agriculture, 2007) The approaches provide a deeper insight into the effects of cooking proce-

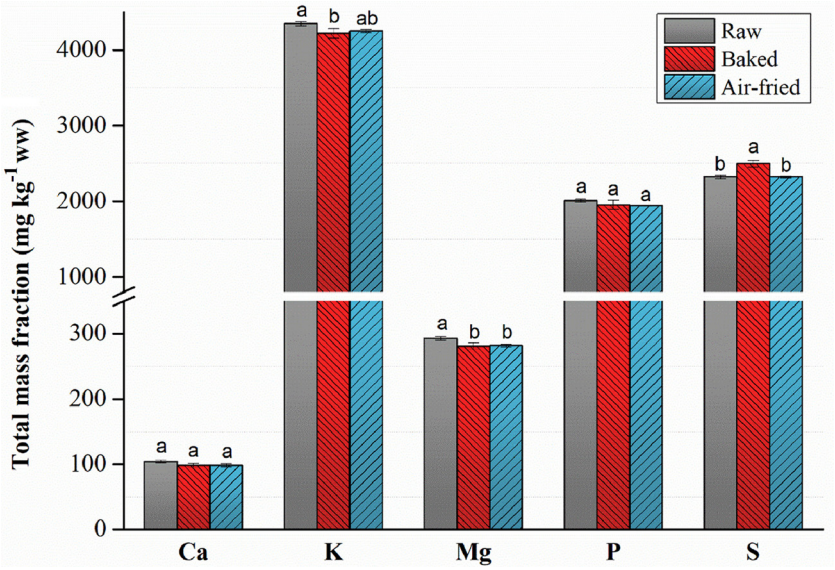

Fig. 1. Total mass fraction of Ca, K, Mg, P and S in raw and cooked *Tilapia* fillet. Bars followed by same letters indicate non-significant differences based on the Tukey test ($p > 0.05$).

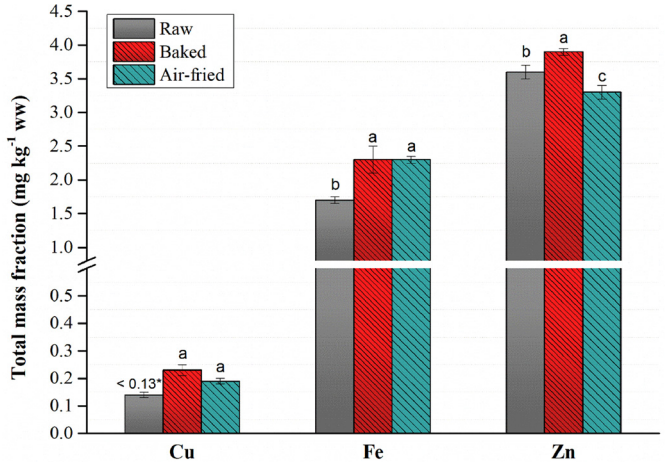

Fig. 2. Total mass fraction of Cu, Fe and Zn in raw and cooked tilapia fillet. Bars followed by same letters indicate non-significant differences based on the Tukey test ($p < 0.05$). * LOQ = 0.13 mg kg^{-1} ww.

dures on the mineral content in food samples. As shown in Table S4, all minerals were almost wholly retained after cooking, as the retention factors obtained in our study ranged from 93 to 100% and from 90 to 100% for baked and air-fried *Tilapia*, respectively. These results confirm that the evaluated cooking procedures did not cause significant losses of essential minerals such as Ca, Cu, Fe, K, Mg, P, S and Zn in *Tilapia* fillets. Comparable to our results, the USDA database for baked or fried seafood samples reported minerals retention factors close to 100% (USDA. U.S. Department of Agriculture, 2007).

3.4. *Validation of the analytical method to estimate the* in vitro *bioaccessibility*

The *in vitro* gastrointestinal chyme is a complex sample containing large amounts of carbon compounds from reagents used for *in vitro* assay, such as pepsin, pancreatin, bile salts and bicarbonate, which may cause severe matrix effects on the elemental determination by ICP-based methods (Serrano et a., 2021). Therefore, we subject the chyme to microwave-assisted digestion. Otherwise, the residual mass (> 0.8 g) was unsuitable for the microwave equipment used in our study (maximum pressure 20 bar). It was acid-digested in a digester block by security, for further mass-balance evaluation.

The instrumental conditions applied to quantify the bioaccessible and residual fractions by SVDV ICP OES were evaluated by addition and recovery experiments using the raw sample. We use Y, Ge, and Bi at a concentration of 2.0 mg L^{-1} as internal standards (IS) to verify the occurrence of eventual transport effects. The analytes were added to the chyme and residue before microwave-assisted and block digestion for the bioaccessible and residual fraction. In both cases, samples were spiked (at 1 level) based on the initial concentration of each analyte found after the previous sample analysis. According to results presented in Table S5 (see Supplementary Material), acceptable recoveries (89–115%) were found, and the IS was not required.

The lack of established validation protocols is a drawback of *in vitro* gastrointestinal digestion assays. In the present study, we evaluated analytical parameters such as LOD and LOQ, measurement precision, matrix effects, and measurement trueness to ensure the quality of the results obtained from the *in vitro* bioaccessibility method. Table S6 (see Supplementary Material) presents the LOD, LOQ, and the measurement precision (% RSD) achieved to estimate the bioaccessible fraction of Ca, Cu, Fe, K, Mg, P, S, and Zn in *Tilapia* fillet. The LOD and LOQ were calculated from the BEC concept, considering the *YF* values, sample mass, and dilution factors used in the *in vitro* assay and sample preparation procedure. LOQs (expressed as wet weight) were suitable for this study, ranging from 0.09 to 30 mg kg^{-1} for Zn and K, respectively. In addition, the measurement precision obtained for all analytes was considered satisfactory (RSD < 7%).

We evaluated the possible occurrence of matrix effects by comparing the slopes of analytical curves prepared from external calibration (EC) and the standard addition method (SAM). Based on the results of *F*-test (Table S7, see Supplementary Material), we apply the *t*-test, assuming equivalent variances ($F_{calculated} < F_{critical}$) for Ca, Mg, S and Zn and different variances ($F_{calculated} > F_{critical}$) for Cu, Fe, K and P. Table S7 shows no statistical differences between the slopes obtained by EC and SAM for all analytes ($t_{calculated} < t_{critical}$) at a 95% confidence level. These results indicate that the matrix constituents did not cause critical effects on the determination of the bioaccessible fraction of Ca, Cu, Fe, K, Mg, P, S, and Zn in *Tilapia* fillets by SVDV ICP OES, and the external calibration may be used for samples analysis.

Additionally, we performed a mass balance based on the sum of bioaccessible plus residual fraction and each analyte's total mass fraction to evaluate the method measurement trueness. The results of the raw, baked, and air-fried cooking procedures are presented in Tables

Table 1
Bioaccessible fraction of essential minerals in raw and cooked Tilapia fillet.

Mineral	Bioaccessible fraction ± standard uncertainty* (mg kg^{-1} ww)		
	In natura	Heat treatments	
		Baking	Air-frying
Ca	41.6 ± 1.0^{a}	43.1 ± 2.7^{a}	46.0 ± 2.0^{a}
Cu	< 0.15**	0.18 ± 0.00^{a}	0.13 ± 0.01^{b}
Fe	0.78 ± 0.03^{b}	1.4 ± 0.0^{a}	1.4 ± 0.0^{a}
K	3772 ± 39^{a}	3640 ± 191^{a}	3758 ± 100^{a}
Mg	188 ± 6^{b}	212 ± 11^{a}	216 ± 4^{a}
P	1621 ± 23^{a}	1607 ± 4^{a}	1582 ± 38^{a}
S	1842 ± 10^{ab}	1964 ± 100^{a}	1727 ± 44^{b}
Zn	0.45 ± 0.00^{c}	1.2 ± 0.1^{a}	0.63 ± 0.04^{b}

* standard deviation (n = 3).
** LOQ = 0.15 mg kg^{-1} ww.Averages followed by equal superscripts letter in the same row indicate non-significant differences by the Tukey test (p > 0.05).

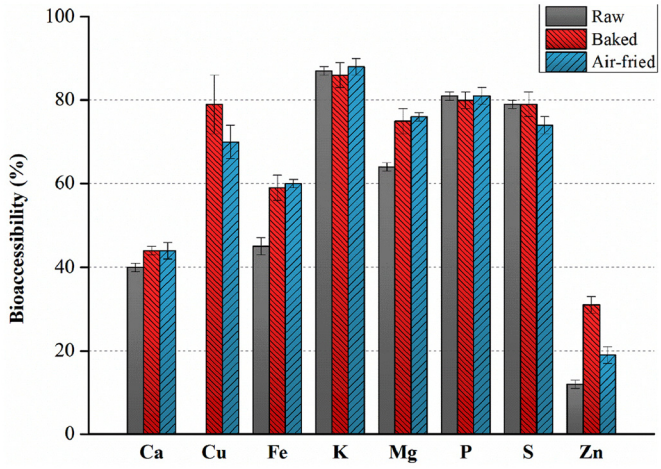

Fig. 3. Bioaccessibility (%) of essential minerals in raw and cooked Tilapia fillet.

S8 and S9, respectively (see Supplementary Material). Except for Cu in the raw sample (values < LOQ), the obtained mass balances were satisfactory for all analytes, ranging from 85 (P) to 107% (Fe) in the raw sample, 85 (Ca) to 102% (Zn) in the baked, and 86 (Ca) to 104% (Cu) in the air-fried. Based on these results, we concluded that the analytical method was reliable for estimating the bioaccessibility of Ca, Cu, Fe, K, Mg, P, S, and Zn in raw and cooked *Tilapia* fillets using SVDV ICP OES.

3.5. Influence of heat treatments on the bioaccessibility of essential minerals

The bioaccessible fraction (expressed as mg kg^{-1}) and bioaccessibility (expressed as a percentage) of Ca, Cu, Fe, K, Mg, P, S and Zn in raw and cooked *Tilapia* are presented in Table 1 and Fig. 3, respectively. In general, bioaccessibilities ranged between 15 and 85%, indicating that the amount of these nutrients ingested from *Tilapia* fillets is not fully available for further absorption by the intestinal epithelium.

Table 1 shows no significant differences (p > 0.05) for the bioaccessible fraction of K, P, and Ca in raw and cooked samples. Fig. 3 shows that most K and P (80–88%) were released from *Tilapia* fillet into the *in vitro* gastrointestinal (GI) chyme. On the other hand, Ca bioaccessibility was less than 45%. Indeed, K and P occur in foods as simple ions and phosphates, respectively, being almost entirely solubilized into the GI tract. In turn, Ca is often found as complex molecules only partially soluble within the intestinal lumen (Hazell, 1985).

The bioaccessible fraction of sulfur in cooked samples did not statistically differ from those found in the raw sample. Still, a slight decrease of S was observed in air-fried *Tilapia* compared to the baking procedure. However, similar bioaccessibilities within the range of 74–79% were found in all cases.

In contrast, the heat treatments significantly influence the bioaccessible fractions of Mg and Fe (p < 0.05). For raw *Tilapia* fillet, 64% of Mg was released into the chyme, and for both cooking procedures, the bioaccessibility increased to 75%. Iron bioaccessibility increased from 45% in the raw sample to approximately 60% after cooking under milder conditions (Fig. 3). This bioaccessibility increase may be related to heat-induced structural and conformational changes of proteins and partial unwinding of the polypeptide chain, which enhance the action of digestive enzymes to cleavage sites and consequent nutrients release (Bhat et al., 2021; Sobral et al., 2018).

The bioaccessible fraction of Cu in the raw sample was below the LOQ (< 0.15 mg kg^{-1} ww), but it was quantified in the cooked samples. As shown in Fig. 3, Cu bioaccessibility was slightly higher after baking the *Tilapia* fillet (79%) than for the air-frying cooking procedure (70%). In seafood, Cu is mainly bound to metallothionein or other complexes stable under moderate temperatures, hampering losses during milder cooking methods (Alves et al., 2018). High bioaccessibility of Cu (85 ± 8%) from *Carp, Yellow croaker,* and *Hairtail* fish species consumed in China was reported with a different *in vitro* digestion model (Wang et al., 2021).

The lowest *in vitro* bioaccessibilities in *Tilapia* fillet were found for Zn. As the solubility of inorganic ions into the GI tract is pH-dependent, the low Zn bioaccessibility may be related to the formation of insoluble Zn compounds at the intestinal pH (Skoryna et al., 1971). Jiang et al. (2021) described a significant decrease in Zn and Fe bioaccessibility from cooked *oysters* due to a possible interaction of the ions and Maillard reaction products (MRPs). Otherwise, our results have shown that the bioaccessible Zn significantly increased in *Tilapia* after cooking. A fraction of approximately 2.5-fold higher than found in the raw sample was released into the *in vitro* chyme in the baked sample. Zinc bioaccessibility increased from 12% (raw) to 19 and 31% for air-fried and baked *Tilapia* fillets, respectively. An increase in Zn bioaccessibility for fish species (*Tuna* and *Plaice*) from the European market after the steaming process was also previously reported (Alves et al., 2018). The partial denaturation of Zn-containing proteins and the exposure of active sites for digestive proteases can be responsible for the greatest bioaccessibility of Zn from cooked *Tilapia* in the present experiment.

3.6. Nutritional issues: supply of essential minerals from Tilapia *consumption*

Following a global trend, seafood consumption has increased in the last few years (Marques et al., 2020). It is suggested that around 1–2 servings (150 g each) of fish per week is recommended as part of a healthy diet for adults (EFSA, 2014). In this sense, we estimated the contribution from *Tilapia* fillets for the recommended daily allowances

Table 2
Estimative of the nutritional contribution from consumption of 150 g of raw and cooked Tilapia fillet.

Mineral	RDA (mg/day) *In natura*		Contribution to RDA (%) Baking in electric oven		Frying in Air-fryer			
	Men	Women	Men	Women	Men	Women	Men	Women
Ca	1000	1000 – 1200*	1.6	1.3 – 1.6	1.5	1.2 – 1.5	1.5	1.2 – 1.5
Cu	0.9	0.9	*n.e.*	*n.e.*	3.8	3.8	3.2	3.2
Fe	8.0	8.0* – 18	3.2	1.4 – 3.2	4.3	1.9 – 4.3	4.3	1.9 – 4.3
K	4700**	4700**	13.9	13.9	13.5	13.5	13.6	13.6
Mg	420	320	10.5	13.7	10.0	13.2	10.1	13.2
P	700	700	43.0	43.0	41.9	41.9	41.6	41.6
Zn	11	8.0	4.9	6.8	5.3	7.3	4.5	6.2

RDA = Recommended Dietary Allowance for adult persons (31–60 years). Values are based on a diet of 2000 calories for men, 1600 calories for women between 31 – 50 years and 1800 calories for women over 51 years old (USDA, 2015).

* RDA for women (> 51 years).

** AI = Adequate Intake (mg/day)*n.e.* = not estimated. Cooper total mass fraction < LOQ.

(RDA) of Ca, Cu, Fe, K, Mg, P, and Zn, considering the consumption of 150 g of raw or cooked sample. Based on the main Brazilian *Tilapia* consumer groups (Baptista et al., 2020), we considered adult persons (men and women) aged between 31 and 60 years using the total mass fraction of each mineral and the dietary requirements recommended by the US Department of Agriculture (USDA. U.S, 2015).

The results (Table 2) suggest that *Tilapia* fillet can be an excellent source of P and K, providing, respectively, more than 40% of RDA and almost 14% of the adequate intake (AI) for these micronutrients. In addition, it can supply about 10 and 13% of Mg RDA to men and women, respectively. On the other hand, 150 g of raw or cooked *Tilapia* fillet consumption provides less than 2% of recommended Ca intake for both adult groups and < 2% of Fe RDA for women aged 30–50. The RDA does not establish values of sulfur.

Overall, in addition to the beneficial effects of cooking processes, such as inhibiting microorganisms' growth and improving organoleptic properties (Sobral et al., 2018), the evaluated heat treatments did not significantly influence the supply of essential minerals from *Tilapia* to the human diet.

4. Conclusion

The total mass and bioaccessible fractions of Ca, Cu, Fe, K, Mg, P, S, and Zn in raw, baked, and air-fried Tilapia fillets after *in vitro* gastrointestinal digestion and microwave-assisted decomposition were measured by SVDV ICP OES. The results obtained from the validation step supported the analytical suitability of the *in vitro* bioaccessibility method for the intended use. Additionally, the SVDV strategy, state of the art in ICP OES instrument, was successfully applied to accurately determine essential minerals from fish in a single instrumental measurement, reducing the analysis time and argon consumption compared to conventional ICP OES with dual or one view system.

By introducing the weight yield factors to express the results, we conclude that both dry-cooking procedures did not influence the total mass fraction of most of the evaluated micronutrients in *Tilapia* fillet, except for Zn, in which a slight reduction of 8% was observed after air-fryer cooking. In turn, the total mass fraction of Fe significantly increased by almost 35% after the two cooking procedures. The *in vitro* bioaccessibility study showed a low release of Zn from the raw sample into the chyme after gastrointestinal digestion. In addition, our results suggest that the baking or air-frying of *Tilapia* fillets can be a way to increase the availability of Fe, Mg, and Zn for intestinal absorption. Based on the estimated RDA values, *Tilapia* fillets can be considered a source of micronutrients such as K, Mg, P, Fe, and Zn in the human diet.

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.

CRediT authorship contribution statement

Herick Macedo Santos: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing – original draft. **Julymar Marcano de Higuera:** Writing – review & editing. **Ana Rita de Araujo Nogueira:** Conceptualization, Resources, Funding acquisition, Supervision, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2022.100080.

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