



Optimization of sample preparation procedures for evaluation of the mineral composition of fish feeds using ICP-based methods



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ABSTRACT

This work describes the application of an experimental design in optimizing the decomposition and spectrometer operational conditions for analysis of fish feeds. The content of 22 elements (six macroelements, four microelements and eleven trace elements) in feeds for fishes (ornamental and for human consumption) was determined by inductively coupled plasma (ICP)-based methods. Limits of quantification were obtained and the values ranged between 0.02 mg kg⁻¹ (Cd) and 76 mg kg⁻¹ (Al). Evaluation of the methods was achieved through analysis of standard reference material, Oyster Tissue (NIST 1566b) and Tea (NCS DC 73351). Element concentrations in feed samples for ornamental fish ranged from < 0.02 mg kg⁻¹ (Cd) to 2.79% (P) and for fish breeding for human consumption from < 0.02 mg kg⁻¹ (Cd) to 2.00% (Ca). The concentrations of the elements obtained in the analyzed fish feeds were within the limits established by Brazilian and American legislation for regulated elements.

1. Introduction

The global market for pet and non-pet animals has grown significantly in the last years. Only in Brazil in 2015, the market reached a total of 132.4 million animals, including dogs, birds, cats, ornamental fish, and reptiles (Brazil, 2014a).

Among domestic animals, Brazil has more than 4000 cataloged ornamental fish species, of which 725 can be marketed for ornamental purposes (Brazil, 2014b). According to Food and Agriculture Organization of the United Nations (FAO), ornamental fish sector is considered one of the most promising segments for generation of work and income (FAO, 2017a). Just in 2013, Brazil exported about 10.5 million US dollars in ornamental fish (Brazil, 2014b). In the same period, Brazilian Institute of Environment and Renewable Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA) estimates that annually around 15 billion US dollars would be generated by ornamental fish production (Brazil, 2016c).

Aquaculture production of fish for human consumption is the fastest growing farming sector and yielded 1.4 billion US dollars in 2015. This growth is being driven by an increasing demand for food. The FAO

report entitled “The State of World Fisheries and Aquaculture 2016 (SOFIA)” predicts that fisheries and aquaculture production in Brazil will grow by approximately 104% by 2025 (Brazil, 2016d; FAO, 2017b).

Monitoring the mineral composition of feeds for ornamental fish and fish for human consumption is of high importance because of the significant contribution of its contents to the corporal functions of the animals and they are relevant for food safety (NRC, 1993; Sales & Janssens, 2003; Mabilia, 2005; Zuanon, Salaro, & Furuya, 2011).

Chemical elements, such as Ca, Cu, Cr, Fe, I, K, Mg, Mn, Na, P, Se, and Zn are essential for growth, bone mineralization, and for metabolism of lipids and carbohydrates, as well as the maintenance of osmotic pressure, muscular relaxation, muscular contraction, control of the general metabolism, formation of sanguine cells, nervous and muscular tissues, and to control the health under stress, among the many body functions. In case of deficiency of these elements the consequences for fishes may include growth decrease, weight loss, bone demineralization, loss of appetite, anemia, cataracts, fins erosions, abnormal caudal fin growth, muscular dystrophy, and thyroid hyperplasia, and others (Sales & Janssens, 2003; Mabilia, 2005; Zuanon, et al., 2011).

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Other chemical elements, such as As, Cd, Pb, and Hg, are considered non-essential for vital functions of fish, but they do not cause problems in their performance. However, when present in excess, these elements can bioaccumulate in the animal bodies and affect human health.

Kwok et al. (2014) found concentration levels of Cd, compatible with bioaccumulation, in muscles and viscera of tilapia (*Oreochromis mossambicus*) and snakehead (*Channa asiatica*), implying potential health risks to two bird species predators, Little Egrets (*Egretta garzetta*) and Chinese Pond Herons (*Ardeola bacchus*).

Nöel, et al. (2013) evaluated the concentrations of As, Cd, Pb, and Hg in muscles of freshwater fish: European eel (*A. Anguilla L.*), bream (*Abramys brama*), common carp (*C. carpio*), European catfish (*Silurus glanis*), roach (*R. rutilus*), perch (*Perca fluviatilis*), pike (*Esox lucius*) and pikeperch (*Sander Lucio-perca*), from five fishing areas in France territory. The concentrations of the elements in fish samples did not exceed the maximum levels established by European legislation, but the found values may be used to estimate the dietary exposure of the general population or high consumers of these products worldwide.

Squadrone et al. (2013) investigated the distribution of As, Cd, Cr, Hg and Pb in muscles, livers, kidneys, and gills of predator European catfish in rivers of Italy. The distribution of the element levels was: As and Hg within muscle > liver ≥ kidney > gill; for Pb was gill > kidney, liver > muscle; for Cd was kidney > gill > muscle; and for Cr order was liver > kidney > muscle > gill. Because of the deleterious effects of these elements in aquatic system and their implications on human health, monitoring and control of the levels of pollution are of great importance.

In Brazil, the Ministry of Agriculture, Livestock, and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento - MAPA) is responsible for regulating foods for pet and non-pet. The Normative Instruction N° 15, May 28, 2009, determines which products must appear on the label, reporting the minimum and maximum values for Ca, and the minimum for the other elements present in fish feeds (Brazil, 2009e). In the United States, the Association of American Feed Control Officials (AAFCO) is responsible for regulating feeds for pets and non-pets and has established a minimum limit of 0.6% of P present in fish feed (AAFCO, 2003).

According to literature reports several spectroanalytical techniques are applied to determine the chemical element contents in feed and fish for consumption. Psoma, Pasiás, Rousis, Barkonikos, & Thomaidis (2014) carried out the determination of As, Cd, Cu, and Hg levels by simultaneous electrothermal atomic absorption spectrometry (SIMAAS) in fish feed for human consumption. Bilandžić et al. (2014) evaluated the concentration of Zn by inductively coupled plasma optical emission spectrometry (ICP OES) in fish samples in Croatia. Silva et al. (2017) determined Cd and Pb levels by ICP OES and Hg by direct mercury analyzer (DMA) in fish consumed in Rio de Janeiro, Brazil. Copat et al. (2013) investigated samples of fish collected in Mediterranean Sea by inductively coupled plasma mass spectrometry (ICP-MS) for determination of As, Cd, Cr, Pb, Mn, Ni, V, and Zn levels. Griboff, Wunderlin, & Monferran (2017) determined the concentrations of Ag, Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sr, V, and Zn by ICP-MS in fish collected in the province of Córdoba, Argentina. Zmozinski et al. (2015) investigated the concentration of As in fish samples from Barcelona, Spain employing direct solid sample analysis (SS) and graphite furnace atomic absorption spectrometry (SS-GF AAS), high-resolution continuum source graphite furnace atomic absorption spectrometry and direct solid sample analysis (HR-CS SS-GF AAS), and high-performance liquid chromatography coupled ICP-MS (HPLC-ICP-MS). Saucedo-Velez, et al. (2017) determined the presence of three organoarsenic compounds, p-arsanilic acid, roxarsone, and nitarosone, in feeds for pig, horse, chicken and rooster by HPLC coupled ultraviolet oxidation hydride generation fluorescence spectrometry (HPLC-UV-HG AFS).

Various experimental designs techniques have been used to optimize the experimental conditions of the above cited methods, promoting a reduction in the number of experiments compared to

univariate methods, as well as an improvement in the analytical parameters, such as accuracy and precision (Novaes, Bezerra et al., 2016; Tuzen et al., 2017; Ma, Wang, Tang and Yang, 2016; Hernandez, Séby, Millour, Noël and Guérin, 2017).

Ávila et al. (2017) applied a full 2⁴ factorial design to find the best conditions for decomposition of wet feed samples for dogs and cats and determined the concentration of Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, P, Sr and Zn by ICP OES. Da Costa, et al. (2013) applied two- and three-levels factorial designs to optimize the sample digestion and operational spectrometer conditions, respectively, and obtained concentrations values for Al, Ca, Cd, Cr, Cu, Ba, Fe, K, Mg, Mn, P, S, Sr, and Zn in dry feed for dogs and cats by ICP OES. Through a two-level factorial design Oliveira, Siqueira, Neto, Coelho, & Alves (2017) optimized the operational parameters of a preconcentration system used for determination of Pb by flame atomic absorption spectrometry (FAAS) in chicken feed samples.

In this context, our work aims to determine the mineral composition of feeds destined for ornamental fishes and human consumption fishes using ICP-based methods, such as ICP OES and ICP-MS, after optimization of the decomposition and spectrometer operational conditions using two- and three-levels factorial designs, respectively.

2. Experimental

2.1. Instrumentation

The samples were homogenized using a planetary ball mill (model PM100, Retsch, Düsseldorf, Germany). The digestions of the feed samples were performed using a microwave oven (model Ethos EZ, Milestone, Sorisole, Italy).

An inductively coupled plasma optical emission spectrometer with an axial view (ICP OES, View Pro, Varian, Mulgrave, Australia) and solid state detector cooled to -5°C (Peltier System) was used to measure the analytes Al II (167.019 nm), B I (249.772 nm), Ca II (317.933 nm), Fe II (259.940 nm), K I (769.897 nm), Mg II (280.270 nm), Mg I (285.213 nm), Mg II (279.553 nm), Mn II (257.610 nm), Na I (588.995 nm), P I (178.222 nm), S II (181.972 nm), and Zn I (213.857 nm), where, (I) is atomic line and (II) is ionic line.

The equipment also has an Echelle polychromator purged with argon gas with a minimum purity of 99.9999% (White Martins, São Paulo, Brazil), which allows measurements in the spectral range between 167 and 785 nm. The conditions used were a Sturman-Masters nebulizer chamber, V-Groove nebulizer, a 2.4-mm injector tube diameter, 1 s signal integration time, sample introduction rate of 0.8 mL min^{-1} , 1.5 L min^{-1} auxiliary gas flow rate, plasma gas flow rate of 15.0 L min^{-1} , radiofrequency power of 1.2 kW and nebulizer gas flow rate of 1.0 L min^{-1} .

Additionally, to perform the chemical analyses of the fish feed samples, it was used an inductively coupled plasma mass spectrometer (ICP-MS, model Thermo X-Series 2, Thermo Scientific, Germany) compound with collision cell technology (CCT). The stable isotopes of ⁴⁵Sc, ⁵¹V, ⁵²Cr, ⁵⁹Co, ⁶⁰Ni, ⁶³Cu, ⁷²Ge, ⁷⁵As, ⁸²Se, ⁸⁸Sr, ¹⁰³Rh, ¹¹¹Cd, ¹²¹Sb, ¹³⁷Ba, ²⁰⁵Tl, and ²⁰⁸Pb were determined. The operational conditions with the standard mode of the spectrometer were optimized using a standard tuning solution of $10\text{ }\mu\text{g L}^{-1}$ for Ba, Ce, Co, In, Li, and U, allowing to obtain the maximum intensity of the mass/load of the isotope (⁷Li, ⁵⁹Co, ¹¹³In, ¹³⁷Ba and ²³⁸U), and to observe the formation of cerium oxide (¹⁴⁰Ce¹⁶O⁺/¹⁴⁰Ce) below 1.5% the double charge (¹⁴⁰Ce²⁺/¹⁴⁰Ce⁺) below 3.0%. An integrated conical nebulizer chamber with a concentric nebulizer was used. For argon plasma generation (minimum purity of 99.998%, White Martins, São Paulo, Brazil), a radio-frequency of 40 MHz with 1.2 kW radio-frequency power, and a nebulizer gas flow rate of 1.0 L min^{-1} were applied. A nickel skimmer was used. To minimize the possibility of molecular or polyatomic spectral interferences for ⁷⁵As and ⁸²Se, the following correction equations were employed: ⁷⁵As = $-3.13220 * ^{77}\text{ArCl}^{+}$ and

$$^{82}\text{Se} = -1.00100 * ^{83}\text{Kr}.$$

2.2. Materials, reagents e solutions

In this work, all reagents used were of analytical grade. Nitric acid 65% (m m^{-1}) and hydrogen peroxide 30% (m m^{-1}) were of suprapure quality (Merck, Darmstadt, Germany). The deionized water for preparation of all solutions was obtained from a Milli-Q purification system (Millipore, Bedford, MA, EUA) to a resistivity of $18.2 \text{ M}\Omega \text{ cm}^{-1}$. Containers used in the experiments were previously decontaminated in a nitric acid solution (10% v v^{-1}) for 24 h (Lopes, Santeli, Oliveira, Carvalho, & Bezerra, 2009; Dantas, Matos, Gouveia, & Lopes, 2013), after which they were washed with deionized water and dried at room temperature.

For ICP OES, external calibration solutions were prepared by proper dilution, according to the working range, from individual stock solutions of 1000 and 4000 mg L^{-1} (Specsol®, São Paulo-SP, Brazil) for Al, B, Ca, Fe, K, Mg, Mn, Na, P, S, and Zn to obtain a working range with concentrations between 5.0 and 400 mg L^{-1} . For ICP-MS analysis, a 100 mg L^{-1} multi-element stock solution containing As, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sb, Se, Sr, and V was used to prepare an intermediate solution at a concentration of 500 $\mu\text{g L}^{-1}$, which was then used to prepare the calibration curve solutions in the concentration range of 0.25 up to 50 $\mu\text{g L}^{-1}$. For the analyses, stable isotopes ^{72}Ge , ^{103}Rh , ^{45}Sc , and ^{205}Tl were added, as internal standards, at a concentration of 50 $\mu\text{g L}^{-1}$ to both calibration curves and samples solutions.

2.3. Preparation of feed fish samples

Eighteen dry feed samples were obtained, fourteen for ornamental fishes and four for fish breeding for human consumption. All samples were from commercial establishments in Salvador and Santo Estevão Cities, Bahia State, Brazil. Dry fish feed samples as granules and flakes are indicated by the manufacturers for the following types of fish: *Acará Bandeira* (*Pterophyllum scalare*), *Acará Disco* (*Symphysodon*), *Barbo Sumatra* (*Puntigrus tetrazona*), *Beijador* (*Helostoma temminckii*), *Betta* (*Betta splendens*), *Carpa* (*Cyprinus carpio*), *Coridora* (*Corydoras*), *Cruzeiro do Sul* (*Hemiodus gracilis*), *Espada* (*Xiphophorus hellerii*), *Guppy* (*Poecilia reticulata*), *Kinguio* (*Carassius auratus*), *Labeo Bicolor* (*Epalzeorhynchus bicolor*), *Mato Grosso* (*Hyphessobrycon eques*), *Molinésia* (*Poecilia latipinna*), *Neon* (*Paracheirodon*), *Oscar Tigre* (*Astronotus ocellatus*), *Paulistinha* (*Brachydanio rerio*), *Platy* (*Xiphophorus maculatus*), *Ramirezi* (*Mikrogeophagus ramirezi*), *Trichogaster lalius* (*Colisa*), *Tricogaster* (*Tricogaster Leeri*) and *Zebra Ciclídeo Africano* (*Haplochromis Zebra Obliquidens*). The dry feed samples for fish production for human consumption in extruded form are suitable for *Curimatã* (*Prochilodus lineatus*), *Tambaqui* (*Colossoma macropomum*) and *Tilápia* (*Pseudocrenilabrinae*).

The samples were manually ground in a mortar and pestle to reduce particle size and then milled in a planetary ball mill with cycles of 10 min and 400 rotations per min (rpm), with a change in the rotational direction every 10 s, ultimately obtaining particles size $\leq 150 \mu\text{m}$. Then, the samples treatment was carried out by microwave-assisted digestion, after optimization of this procedure through a fractional factorial design.

The optimized procedure consisted of approximately 0.1000 g of feed fish measured and transferred to polytetrafluoroethylene (PTFE) tubes. Seven-hundred microliters of HNO_3 (65% m m^{-1}) were then added, also 1.65 mL of H_2O_2 (30% m m^{-1}) and 7.65 mL of deionized water (to a final volume of 10 mL). The PTFE tubes were submitted to a temperature of 170 °C for 20 min under a microwave power of 400 W. After the procedure, the samples were transferred to 50 mL Falcon tubes filled up to a 15 mL volume with deionized water.

All procedures were performed in triplicate, including blank solutions and standard reference materials. The ICP OES analysis was performed directly on the digested samples. For ICP-MS analysis, the

samples were diluted using a dilution factor of 3:10.

2.4. Quality control

Accuracy and precision of the ICP-based methods were evaluated by analysis of standard reference materials (SRM) of Oyster Tissue (NIST 1566b) acquired from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and Tea (NCS DC 73351) obtained through China National Analysis Center for Iron and Steel (Beijing, China).

2.5. Optimization of experimental conditions

A 2^{5-2} factorial design was used to optimize the microwave-assisted digestion conditions. The design variables chosen were: HNO_3 and H_2O_2 concentrations, temperature, digestion time, and microwave radiation power. The response for the design was evaluated using the concept of multiple response (MR), obtained from concentrations of Cu, Fe, Mg, and Mn in a commercial sample of ornamental fish feed (Ferreira, 2015; Ferreira, et al., 2017; Novaes, Ferreira, et al., 2016).

To assess the operating conditions of the inductively coupled plasma optical emission spectrometer (ICP OES), a Doehlert design for two variables was applied for nebulizer gas flow rate (NG flow rate: 0.6–1.0 L min^{-1}) and radio frequency power (RF power: 1.0–1.4 kW), achieving as response the ratio of the emission intensities of ionic and atomic magnesium lines [Mg II (280.265 nm)/Mg I (285.213 nm)] corresponding to a plasma robustness condition during the introduction of the solutions (Mermert, 1991).

A simultaneous experimental design was performed using triplicate the central point to evaluate the experimental error of design. The experimental data were processed using Statistica® software version 6.0.

3. Results and discussion

3.1. Optimization of decomposition conditions of the feed fish

A 2^{5-2} factorial design with triplicate of the central point was used to identify the best decomposition conditions to analyze dry feed samples for fishes for ornamental and human consumption purposes (Novaes, Bezerra et al., 2016). The variables evaluated were the concentrations of HNO_3 (1.0–4.0 mol L^{-1}) and H_2O_2 (1.0–5.0% m m^{-1}), temperature (170–210 °C), digestion time (10–20 min) and microwave radiation power (400–800 W) as shown in Table 1 and Table S1, in Supplementary material.

The experiments were performed in random order. The triplicate of the central point was used to estimate the experimental error of the factorial design. A dry mass of approximately 0.10 g of the commercial ornamental fish feed was used in all experiments. After the application of the design, the concentrations of Cu, Fe, Mg, and Mn were obtained for each experiment of the proposed design and used to calculate the MR. These data were subsequently used to determine the suitable conditions for an efficient and simultaneous decomposition for all elements. The MR value was calculated according to Eq. (1) (Santos, et al., 2009; Da Costa, et al., 2013; Ferreira, 2015).

$$\text{MR} = \frac{\text{Conc. Cu}}{\text{Máx. Conc. Cu}} + \frac{\text{Conc. Fe}}{\text{Máx. Conc. Fe}} + \frac{\text{Conc. Mg}}{\text{Máx. Conc. Mg}} + \frac{\text{Conc. Mn}}{\text{Máx. Conc. Mn}} \quad (1)$$

where “Conc.Cu” is copper concentrations found in each experiment and the “Max.Conc.Cu” is the maximum concentrations of copper found in the factorial design. The same logic applies to the concentrations of Fe, Mg, and Mn.

After application of simultaneous analysis of the responses, the variables and the interactions causing a significant variation in the MR were identified. The evaluation of the proposed fractional factorial

Table 1Matrix of the 2^{5-2} fractional factorial design with triplicate of the central point used in the evaluation of the conditions for sample digestion assisted by microwave radiation.

| Experiments | Coded values/Real values | | | | | Multiple response |
|-------------|--|---|---------------------|----------------------------------|---------------|-------------------|
| | HNO ₃ (mol L ⁻¹) | H ₂ O ₂ % (m m ⁻¹) | Temperature (°C) | Microwave radiation power (W) | Time (min) | |
| 1 | (-1)/1.0 | (-1)/1.0 | (-1)/170 | (1)/800 | (1)/20 | 3.61 |
| 2 | (1)/4.0 | (-1)/1.0 | (-1)/170 | (-1)/400 | (-1)/10 | 3.35 |
| 3 | (-1)/1.0 | (1)/5.0 | (-1)/170 | (-1)/400 | (1)/20 | 3.82 |
| 4 | (1)/4.0 | (1)/5.0 | (-1)/170 | (1)/800 | (-1)/10 | 3.32 |
| 5 | (-1)/1.0 | (-1)/1.0 | (1)/210 | (1)/800 | (-1)/10 | 2.64 |
| 6 | (1)/4.0 | (-1)/1.0 | (1)/210 | (-1)/400 | (1)/20 | 3.49 |
| 7 | (-1)/1.0 | (1)/5.0 | (1)/210 | (-1)/400 | (-1)/10 | 3.92 |
| 8 | (1)/4.0 | (1)/5.0 | (1)/210 | (1)/800 | (1)/20 | 3.25 |
| 9 | (0)/2.5 | (0)/3.0 | (0)/190 | (0)/600 | (0)/15 | 3.73 |
| 10 | (0)/2.5 | (0)/3.0 | (0)/190 | (0)/600 | (0)/15 | 3.64 |
| 11 | (0)/2.5 | (0)/3.0 | (0)/190 | (0)/600 | (0)/15 | 3.68 |

design was achieved through resolution III, a type of resolution that does not confound the main effects with other main effects, but confounds the main effects with the effects of two-factor interactions (Ferreira, 2015). The Pareto chart obtained for the fractional factorial design is shown in Fig. 1.

The Pareto chart shows the contrast effects for HNO₃ and H₂O₂ concentrations, temperature, microwave radiation power, and digestion time, using the MR as the significant response. The magnitude of the effects is represented by columns, while the vertical line indicates the statistical significance at $p \geq 0.05$.

Fig. 1 shows that the effects of the contrasts for microwave radiation power, HNO₃, and H₂O₂ concentrations, time, and temperature were significant for digestion. However, the most significant variable was microwave radiation power, followed by H₂O₂ concentration, digestion time, temperature and HNO₃ concentration. An increase in the MR is observed at lower concentration of HNO₃, microwave radiation power and temperature, and at higher concentration of H₂O₂ and digestion time.

In all experiments, the efficiency in the decomposition of the fish feed sample (e.g., composition of bran, vesicles, meats, cereals) was increased using diluted HNO₃, whose oxidizing power is lower, due to the addition of H₂O₂, which promotes de regeneration of HNO₃ contributing to the maintenance of the acid concentration in the pressurized system and favoring the oxidation of the organic matter. That is why higher concentration of H₂O₂ improves the efficiency in the decomposition of the sample even with low concentrations of HNO₃ (Bizzi, Flores, Picoloto, Barin, & Nóbrega, 2010). This procedure is in

line with the principles of Green Chemistry.

The curvature test was applied to the obtained responses in the proposed fractional factorial design. This test was calculated using the following expression: curvature = MREx - MCP, where MREx is the average of the responses of eight specified experiments, and MCP is the average of the responses obtained by triplicate of the central point. Analysis of the results, MREx = (3.43) and MCP = (3.68), suggests a negative value (-0.21), meaning that there is a maximum condition in the region of the central point of the experimental dominions established for the proposed design (Table 1) (Ferreira, 2015).

After simultaneous analysis of the data, it was possible to establish the digestion procedure of Experiment 3 as an optimal condition. The variables in this experiment are used at the levels indicated by the Pareto chart and the MR (3.82) is not significantly different from the highest obtained response, Experiment 7, whose MR was 3.92. Thus, in subsequent experiments the following conditions were used: 1.0 mol L⁻¹ of HNO₃, 5% m m⁻¹ of H₂O₂, 170 °C for digestion temperature, 20 min digestion time, and 400 W microwave radiation power, for a reaction volume of 10 mL and about 0.10 g of fish feed sample (ornamental and for human consumption).

3.2. Evaluation of spectrometer operating conditions through Doehlert design

Evaluation of plasma robustness, experimental conditions of radio-frequency (RF) power and nebulizer gas flow rate (NG flow), was performed through a Doehlert design, as shown in Table 2. The response of the proposed design was assessed through the ratio of the emission intensities of magnesium lines (Mg II [280.265 nm] and Mg I [285.213 nm]), whose ratio ≥ 8 indicates a robust condition, appropriate for the introduction of a solution into the plasma, with no loss of the energy efficiency (Silva, Lena, Froes, Costa, & Nascentes, 2012; Dennaud, Howes, Poussel, & Mermet, 2001).

This design was applied to a digested solution of a commercial ornamental fish feed and a standard multi-element (macroelements, microelements, and trace elements) in HNO₃ (1.0 mol L⁻¹) solution medium. Simultaneous evaluation of the responses found for magnesium emission ratios, obtained from the intensities for the two solutions, was achieved by the MR concept calculated according to Eq. (2) (Ferreira, 2015).

$$MR = \left(\frac{MgII/MgI}{(MgII/MgI)_{max}} \right)_{digest} + \left(\frac{MgII/MgI}{(MgII/MgI)_{max}} \right)_{standard} \quad (2)$$

where, (Mg II/Mg I) is the value of the relationship between the intensities of the emission lines [Mg II 280.265 nm and Mg I 285.208 nm] and (Mg II/Mg I)_{max} is the maximum value of the relationship between the emission intensity lines of magnesium for the digested commercial feed sample (7.30) and multi-element solution (7.76)

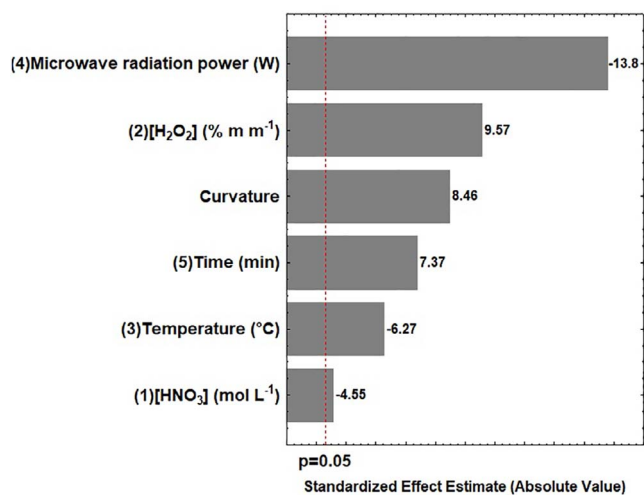


Fig. 1. Pareto chart obtained for the 2^{5-2} fractional factorial design used to evaluate the conditions of microwave-assisted samples digestion.

Table 2
Matrix of the Doehlert design for optimization of the operational conditions of the spectrometer.

| Experiments | Coded values/Real values Responses | | | | |
|-------------|------------------------------------|------------------|---|---|-----------------------------------|
| | NG flow (L min ⁻¹) | RF power (kW) | ^a (Mg II/Mg I) Ratio ^a | ^b (Mg II/Mg I) Ratio ^b | ^c Multiple response |
| 1 | (+1)/1.0 | (0)/1.2 | 7.13 | 7.72 | 1.97 |
| 2 | (+0.5)/0.9 | (+0.866)/1.4 | 7.30 | 7.76 | 2.00 |
| 3 | (-1)/0.6 | (0)/1.2 | 3.64 | 3.47 | 0.95 |
| 4 | (-0.5)/0.7 | (-0.866)/1.0 | 3.01 | 2.96 | 0.79 |
| 5 | (+0.5)/0.9 | (-0.866)/1.0 | 5.94 | 6.27 | 1.62 |
| 6 | (-0.5)/0.7 | (+0.866)/1.4 | 5.22 | 5.03 | 1.36 |
| 7 | (0)/0.8 | (0)/1.2 | 5.90 | 6.05 | 1.59 |
| 8 | (0)/0.8 | (0)/1.2 | 5.89 | 6.02 | 1.58 |
| 9 | (0)/0.8 | (0)/1.2 | 5.94 | 6.02 | 1.59 |

^a (Mg II/Mg I) ratios were obtained through (a) digested commercial sample of feed fish; (b) multielement solution in acid medium.

^c Multiple response was calculated according to Eq. (2).

After analysis of the proposed Doehlert design, Pareto chart was generated to evaluate which variables would be significant for the system according to Fig. S1, in Supplementary material. The effects of the evaluated conditions for RF power and NG flow rate were significant for both, linear and quadratic variables and linear interaction (Fig. S1, in Supplementary material).

The most significant variables were the NG flow rate, followed by the RF power, both for maximum values (i.e., when these variables are at higher levels, the MR obtained is maximized). The quadratic variables and the linear interaction between RF power and NG flow rate were significant for the minimum values.

The contour chart (Fig. S2, in Supplementary material) shows that, increasing NG flow rate and RF power to the maximum levels, the obtained response gets near to the optimum region of the experiment. The experiment presenting the best response in the three situations was Experiment 2, where a RF power of 1.4 kW and a NG flow rate of 0.9 L min⁻¹, with a value ratio (Mg II/Mg I) of 7.30 for digested sample solution, and of 7.76 for multi-element solution in HNO₃ 1.0 mol L⁻¹. Under these conditions the MR value was 2.00, which is not considered a plasma robustness condition. However, since a V-Groove pneumatic nebulizer and a Sturman-Masters nebulizer chamber were applied for the analysis, it was not expected that these experiments could achieve a ratio value ≥ 8.0 (Trevizan, Vieira, Nogueira, & Nóbrega, 2005; Sharp, 1988a; Sharp, 1988b).

The V-Groove nebulizer has higher tolerance to dissolved solids. However, its design is not suitable for generating a homogeneous aerosol, instead promotes irregular particle size with low efficiency. Therefore, to minimize this disadvantage, an increasing in the sample pumping flow is recommend. The Sturman-Masters chamber allows higher particles to be transported, forming larger drops of the aerosol, affecting the desolvation process and the line of analytes, thus resulting in the lowest intensity and affecting the sensitivity (Trevizan, et al., 2005; Sharp, 1988a; Sharp, 1988b).

After simultaneous analysis of the data, the critical values obtained (Table S2, in Supplementary material) were RF power of 1.219 kW and NG flow rate of 1.2 L min⁻¹. However, the obtained NG flow rate value was outside the proposed experimental domain. Therefore, a visual inspection of the contour chart revealed that a RF power of 1.2 kW and NG flow rate of 1.0 L min⁻¹ is an optimal condition, the same of the Experiment 1 of the proposed design. In this experiment, Mg II/Mg I obtained ratios were 7.13, for digested feed fish sample solution, and 7.72 for multi-element solution in HNO₃ 1.0 mol L⁻¹, with a value of 1.97 for the MR, the second higher robustness condition.

The results were evaluated by a linear regression method between predicted and experimental values (Fig. S3, in Supplementary material). The Equation obtained was $y = (0.9766 \pm 0.0567)x + (0.1298 \pm 0.3241)$ and the correlation coefficient (r) was 0.9942. As the angular coefficient includes the unit value

(0.9199 < 1.0000 < 1.0333) and linear coefficient includes the zero value (-0.1943 < 0.0000 < 0.4539), it can be concluded that the experimental and predicted values, for angular and linear coefficients, are within the acceptance range for the ideal condition of linear regression analysis (a = 1 and b = 0). The correlation coefficient (0.9942) was greater than 0.99; an acceptable correlation between the predicted and experimental values (Ferreira, 2015; Nechar, Molina, & Bosque-Sendra, 1999).

3.3. Validation of the ICP-based methods proposed

For ICP OES method, limit of detection (LoD) and limit of quantification (LoQ) were calculated using the background equivalent concentration (BEC) and signal-to-background ratio (SBR), according to International Union of Pure and Applied Chemistry (IUPAC), where $BEC = C_{\text{standard}}/SBR$, with $SBR = (I_{\text{standard}} - I_{\text{blank}})/I_{\text{blank}}$, and C_{standard} is the reference element concentration in the solution, I_{standard} and I_{blank} are the emission intensities for the reference element and blank solutions, respectively, at the selected wavelength (IUPAC, 1978).

The LoD was then calculated as $(3 \times RSD_{\text{blank}} \times BEC/100)$ and LoQ expressed as $(10 \times RSD_{\text{blank}} \times BEC/100)$, where RSD_{blank} is the relative standard deviation of the emission intensity measurements for the blank solution at the selected wavelength (IUPAC, 1978; Schiavo, Trevizan, Filho, & Nóbrega, 2009).

Table 3 shows the obtained values of LoD and LoQ for feed fish samples analyzed by ICP OES, which ranged between 0.2 and 23 mg kg⁻¹ for LoD and between 0.7 and 76 mg kg⁻¹ for LoQ, for the elements Mn and Al, respectively.

In ICP-MS analysis, LoD and LoQ were calculated as 3- and 10-times the standard deviation of the measurements of the blank solution, respectively, divided by the slope of the equation obtained from calibration curve for each element. The obtained values for LoD varied between 0.005 mg kg⁻¹ (Cd) and for LoQ between 0.1 mg kg⁻¹ (Cr) and between 0.02 mg kg⁻¹(Cd) and 0.7 mg kg⁻¹(Cr), respectively (IUPAC, 1978).

Psoma, et al. (2014) reported LoDs in samples of fish for human consumption of 6.4 µg kg⁻¹ (Cd), 0.08 mg kg⁻¹ (Pb), 0.52 mg kg⁻¹ (As) and 0.102 mg kg⁻¹ (Cu) by SIMAAS, and 2.4 µg kg⁻¹ (Cd), 0.0304 mg kg⁻¹ (Pb), 5.2 µg kg⁻¹ (As) and 0.056 mg kg⁻¹ (Cu) by GF AAS. Zmozinski et al. (2015) reported LoD values of 0.1 µg kg⁻¹ (As) by SS-GF AAS and 0.05 µg kg⁻¹ by HR-CS SS-GF AAS, for marine fish samples. Silva et al. (2017) reported LoD values of 2.02 µg kg⁻¹ (As), 0.11 µg kg⁻¹ (Cd) and 1.9 µg kg⁻¹ (Pb) by ICP OES, also for marine fish samples.

The accuracy of the proposed ICP-based methods was confirmed through analysis of standard reference materials (SRM), Oyster tissue (NIST 1566b) and Tea (NCS DC 73351). The agreement between the certified values and those obtained for the samples ranged from

Table 3
Results obtained for analysis of standard reference materials by ICP OES and ICP-MS, as well as the LoD and LoQ values.

| | SRM NIST 1566b | | | | | | | SRM NCS DC 73351 | | | | | | | Parameters ^b | | |
|--------------------------|---------------------------|-----------------|-----------------|---------------|--------------------------|---------------------------|--------------------|------------------|---------------|---------|----------------------------|----------------------------|--|--|-------------------------|--|--|
| | Element | Certified value | Found value | Agreement (%) | RSD (%) | Element | Certified value | Found value | Agreement (%) | RSD (%) | LoD (mg kg ⁻¹) | LoQ (mg kg ⁻¹) | | | | | |
| ICP OES | Al (mg kg ⁻¹) | 197.2 ± 6.0 | 220.9 ± 31.1 | 112 ± 14 | 5.7 | Al (%) | 0.30 ^a | 0.26 ± 0.01 | 87 ± 2 | 0.7 | 23 | 76 | | | | | |
| | B (mg kg ⁻¹) | 4.5 ± 1.9 | < 3.3 | n.d. | n.d. | B (mg kg ⁻¹) | 15 ± 4 | 12 ± 1 | 80 ± 7 | 3.4 | 1.0 | 3.3 | | | | | |
| | Ca (%) | 0.0838 ± 0.0020 | 0.0876 ± 0.0051 | 104 ± 6 | 2.3 | Ca (%) | 0.43 ± 0.04 | 0.43 ± 0.02 | 100 ± 4 | 1.7 | 17 | 57 | | | | | |
| | Fe (mg kg ⁻¹) | 205.8 ± 6.8 | 175.6 ± 11.9 | 85 ± 6 | 2.7 | Fe (mg kg ⁻¹) | 264 ± 15 | 218 ± 49 | 83 ± 19 | 9.1 | 5.3 | 18 | | | | | |
| | K (%) | 0.652 ± 0.009 | 0.560 ± 0.009 | 86 ± 1 | 0.7 | K (%) | 1.66 ± 0.12 | 1.41 ± 0.05 | 85 ± 3 | 1.3 | 12 | 40 | | | | | |
| | Mg (%) | 0.1085 ± 0.0023 | 0.1139 ± 0.0011 | 105 ± 1 | 0.4 | Mg (%) | 0.17 ± 0.02 | 0.18 ± 0.01 | 107 ± 4 | 1.4 | 2.6 | 8.7 | | | | | |
| | Mn (mg kg ⁻¹) | 18.5 ± 0.2 | 14.7 ± 1.2 | 81 ± 3 | 1.3 | Mn (mg kg ⁻¹) | 1240 ± 70 | 1236 ± 31 | 99 ± 2 | 1.0 | 0.2 | 0.7 | | | | | |
| | Na (%) | 0.3297 ± 0.0053 | 0.3296 ± 0.0443 | 100 ± 13 | 5.4 | Na (mg kg ⁻¹) | 44 ± 6 | < 53 | n.d. | n.d. | 16 | 53 | | | | | |
| | P (%) | n.i. | 0.885 ± 0.149 | n.d. | 6.8 | P (mg kg ⁻¹) | 2840 ± 90 | 2385 ± 100 | 84 ± 4 | 1.7 | 11 | 37 | | | | | |
| | S (%) | 0.689 ± 0.014 | 0.662 ± 0.003 | 99 ± 14 | 5.7 | S (%) | 0.245 ± 0.022 | 0.223 ± 0.006 | 91 ± 2 | 1.1 | 15 | 50 | | | | | |
| | Zn (mg kg ⁻¹) | 1424 ± 46 | 1394 ± 99 | 102 ± 14 | 5.7 | Zn (mg kg ⁻¹) | 26.3 ± 2.0 | 29.3 ± 2.9 | 111 ± 11 | 4.0 | 1.2 | 4.6 | | | | | |
| ICP-MS | As (mg kg ⁻¹) | 7.65 ± 0.65 | 8.54 ± 0.68 | 112 ± 9 | 3.2 | As (mg kg ⁻¹) | 0.28 ± 0.04 | 0.29 ± 0.03 | 105 ± 9 | 3.7 | 0.01 | 0.03 | | | | | |
| | Ba (mg kg ⁻¹) | 8.6 ± 0.3 | 7.7 ± 0.2 | 90 ± 3 | 1.3 | Ba (mg kg ⁻¹) | 58 ± 6 | 64 ± 3 | 110 ± 5 | 2.1 | 0.05 | 0.2 | | | | | |
| | Cd (mg kg ⁻¹) | 2.48 ± 0.08 | 2.19 ± 0.22 | 88 ± 9 | 4.1 | Cd (mg kg ⁻¹) | 0.057 ± 0.010 | 0.056 ± 0.001 | 98 ± 2 | 0.8 | 0.005 | 0.02 | | | | | |
| | Co (mg kg ⁻¹) | 0.371 ± 0.009 | 0.333 ± 0.043 | 90 ± 12 | 5.3 | Co (mg kg ⁻¹) | 0.18 ± 0.02 | 0.19 ± 0.03 | 108 ± 14 | 5.3 | 0.008 | 0.03 | | | | | |
| | Cr (mg kg ⁻¹) | n.i. | 0.950 ± 0.142 | n.d. | 6.0 | Cr (mg kg ⁻¹) | 0.80 ± 0.03 | 0.83 ± 0.20 | 104 ± 25 | 10 | 0.2 | 0.7 | | | | | |
| | Cu (mg kg ⁻¹) | 71.6 ± 1.6 | 59.1 ± 5.4 | 83 ± 8 | 3.7 | Cu (mg kg ⁻¹) | 17.3 ± 1.8 | 15.8 ± 3.9 | 91 ± 22 | 9.9 | 0.06 | 0.2 | | | | | |
| | Ni (mg kg ⁻¹) | 1.04 ± 0.09 | 0.89 ± 0.27 | 86 ± 4 | 1.7 | Ni (mg kg ⁻¹) | 4.6 ± 0.5 | 4.8 ± 0.4 | 103 ± 9 | 3.6 | 0.04 | 0.1 | | | | | |
| | Pb (mg kg ⁻¹) | 0.308 ± 0.009 | 0.298 ± 0.086 | 103 ± 12 | 4.7 | Pb (mg kg ⁻¹) | 4.4 ± 0.03 | 4.4 ± 0.2 | 100 ± 5 | 3.4 | 0.01 | 0.03 | | | | | |
| | Sb (mg kg ⁻¹) | 0.011 ± 0.002 | < 0.03 | n.d. | 7.3 | Sb (mg kg ⁻¹) | 0.056 ± 0.006 | 0.063 ± 0.001 | 112 ± 2 | 0.7 | 0.01 | 0.03 | | | | | |
| | Se (mg kg ⁻¹) | 2.06 ± 0.15 | 2.46 ± 0.19 | 120 ± 9 | 3.0 | Se (mg kg ⁻¹) | 0.072 ^a | < 0.07 | n.d. | n.d. | 0.02 | 0.07 | | | | | |
| | Sr (mg kg ⁻¹) | 6.8 ± 0.2 | 7.0 ± 0.3 | 103 ± 5 | 1.9 | Sr (mg kg ⁻¹) | 15.2 ± 0.7 | 12.2 ± 1.6 | 81 ± 11 | 10 | 0.1 | 0.3 | | | | | |
| V (mg kg ⁻¹) | 0.577 ± 0.023 | 0.475 ± 0.044 | 82 ± 8 | 3.8 | V (mg kg ⁻¹) | 0.86 ^a | < 0.03 | n.d. | n.d. | 0.008 | 0.03 | | | | | | |

All results were expressed as mean ± 95% confidence interval (n = 3).

n.i. = not informed.

n.d. = not determined.

^a Informed value in SRM certificate.

^b Values calculated from volume of 15 mL and mass of 0.1000 g of fish feed sample.

$80 \pm 7\%$ (B) up to $120 \pm 9\%$ (Se). The precision was expressed as relative standard deviation (RSD%, $n = 3$), which was better than 10%. Thus, the proposed analytical methods were considered accurate and precise for the determination of macroelements (Ca, K, Na, Mg, P and S), microelements (Cu, Fe, Mn and Zn), and trace elements (Al, As, Ba, Cd, Co, Cr, Ni, Sb, Se, Sr, Pb, and V) present in fish feed samples for ornamental purpose and for human consumption, as shown in Table 3.

3.4. Analytical application of the analytical methods in fish feed

The optimized analytical methods were applied to analysis of 18 dry fish feed samples: 14 samples for ornamental fish (OF) and four samples for fish for human consumption (CF). In these samples, concentrations of macroelements (Ca, K, Na, Mg, P, and S), microelements (Cu, Fe, Mn and Zn), and trace elements (Al, As, Ba, Cd, Co, Cr, Ni, Sb, Se, Sr, Pb, and V) were determined employing ICP OES and ICP-MS.

The element concentrations in ornamental fish feed samples ranged from 0.014 ± 0.001 to $0.341 \pm 0.012\%$ (Al), < 3.3 to $20 \pm 1.5 \text{ mg kg}^{-1}$ (B), 0.162 ± 0.014 to $3.37 \pm 0.36\%$ (Ca), 136 ± 14 to $954 \pm 73 \text{ mg kg}^{-1}$ (Fe), 0.628 ± 0.062 to $1.82 \pm 0.32\%$ (K), 15.0 ± 0.7 to $180 \pm 19 \text{ mg kg}^{-1}$ (Mn), 0.147 ± 0.016 to $0.287 \pm 0.009\%$ (Mg), 0.213 ± 0.003 to $1.15 \pm 0.11\%$ (Na), 0.355 ± 0.020 to $2.79 \pm 0.38\%$ (P), 0.177 ± 0.003 to $0.890 \pm 0.200\%$ (S), 31.0 ± 1.3 to $296 \pm 9 \text{ mg kg}^{-1}$ (Zn), 0.131 ± 0.034 to $3.52 \pm 0.50 \text{ mg kg}^{-1}$ (As), 3.52 ± 0.26 to $43.0 \pm 2.1 \text{ mg kg}^{-1}$ (Ba), < 0.02 to $0.331 \pm 0.014 \text{ mg kg}^{-1}$ (Cd), 0.158 ± 0.009 to $17.0 \pm 3.7 \text{ mg kg}^{-1}$ (Co), 0.818 ± 0.210 to $2.83 \pm 0.15 \text{ mg kg}^{-1}$ (Cr), 4.40 ± 0.66 to $45.0 \pm 12.0 \text{ mg kg}^{-1}$ (Cu), 0.633 ± 0.112 to $2.08 \pm 0.30 \text{ mg kg}^{-1}$ (Ni), 0.147 ± 0.006 to $1.35 \pm 0.26 \text{ mg kg}^{-1}$ (Pb), < 0.03 to $0.078 \pm 0.011 \text{ mg kg}^{-1}$ (Sb), < 0.3 to $2.89 \pm 0.53 \text{ mg kg}^{-1}$ (Sr), 6.67 ± 0.08 to $50 \pm 3.1 \text{ mg kg}^{-1}$ (Sr), and 0.308 ± 0.045 to $1.52 \pm 0.23 \text{ mg kg}^{-1}$ (V). This complete information is shown in Tables S3 and S4 in Supplementary material.

Based on the statistical summary obtained for the element concentrations in ornamental fish feed samples (Table 4), the decreasing order of elements concentrations was as following: Ca > P > K > Na > S > Mg > Al > Fe > Zn > Mn > Cu > Sr > Ba > B > Co > Cr > Ni > As > Se > V > Pb > Cd > Sb. Macroelements

Table 4

Statistical summary of the concentrations of inorganic constituents in ornamental fish feed samples.

| Element | Mean | Median | Minimum | Maximum | n |
|----------------------------|-------|--------|---------|---------|----|
| Al (%) | 0.127 | 0.074 | 0.014 | 0.341 | 14 |
| B (mg kg^{-1}) | 14.0 | 18.0 | 6.24 | 20.0 | 5 |
| Ca (%) | 1.60 | 1.44 | 0.162 | 3.37 | 14 |
| Fe (mg kg^{-1}) | 521 | 465 | 136 | 954 | 14 |
| K (%) | 1.08 | 1.01 | 0.63 | 1.82 | 14 |
| Mg (%) | 0.214 | 0.208 | 0.147 | 0.285 | 14 |
| Mn (mg kg^{-1}) | 92.0 | 79.0 | 15.0 | 180 | 13 |
| Na (%) | 0.533 | 0.608 | 0.170 | 1.15 | 14 |
| P (%) | 1.31 | 1.15 | 0.355 | 2.79 | 14 |
| S (%) | 0.456 | 0.443 | 0.177 | 0.890 | 14 |
| Zn (mg kg^{-1}) | 151 | 151 | 31.0 | 296 | 14 |
| As (mg kg^{-1}) | 1.23 | 1.05 | 0.131 | 3.52 | 14 |
| Ba (mg kg^{-1}) | 17.0 | 12.0 | 3.52 | 43.0 | 14 |
| Cd (mg kg^{-1}) | 0.156 | 0.163 | 0.050 | 0.331 | 13 |
| Co (mg kg^{-1}) | 8.46 | 8.42 | 0.158 | 17.0 | 14 |
| Cr (mg kg^{-1}) | 1.54 | 1.46 | 0.818 | 2.83 | 14 |
| Cu (mg kg^{-1}) | 19.0 | 20.0 | 4.40 | 45.0 | 14 |
| Ni (mg kg^{-1}) | 1.28 | 1.28 | 0.633 | 2.08 | 14 |
| Pb (mg kg^{-1}) | 0.652 | 0.482 | 0.147 | 1.35 | 14 |
| Sb (mg kg^{-1}) | 0.056 | 0.055 | 0.038 | 0.078 | 9 |
| Se (mg kg^{-1}) | 1.13 | 0.925 | 0.528 | 2.89 | 13 |
| Sr (mg kg^{-1}) | 18.0 | 17.0 | 6.67 | 50.0 | 14 |
| V (mg kg^{-1}) | 0.905 | 0.804 | 0.308 | 1.52 | 14 |

n = number of fish feed samples using statistical summary.

(Ca, P, K, Na, S and Mg) are present in higher concentrations, followed by the microelements (Fe, Zn, Mn and Cu). Trace elements are present at lowest concentrations, except Al, whose concentrations were higher than the microelements in this type of feed.

Comparing the determined average concentrations with the values expressed in the manufacturer labels for Cu (6.80 mg kg^{-1}), Fe (68.0 mg kg^{-1}), Mn (54.4 mg kg^{-1}), Se (0.20 mg kg^{-1}), and Zn (68.0 mg kg^{-1}), it was found that these are 2.8-, 7.7-, 1.5-, 5.5-, and 2.2-times, respectively, lower than those determined, therefore in agreement with the minimum values reported. Comparing the obtained values, according to what is required by MAPA (Brazilian legislation), the mean Ca concentrations (1.60%) are below what is reported on the product labels, which expressed a minimum mean concentration of 1.65% and maximum of 2.92%. For P, the minimum mean concentration reported on the product labels was 0.81%, and it was found 1.31%, 1.62 times higher than reported by the manufacturers.

The element concentrations present in the feed for fish for human consumption ranged from < 76 to $0.036 \pm 0.002\%$ (Al), 0.787 ± 0.195 to $2.00 \pm 0.05\%$ (Ca), < 18 to $542 \pm 27 \text{ mg kg}^{-1}$ (Fe), 0.681 ± 0.066 to $0.876 \pm 0.072\%$ (K), 17.0 ± 1.9 to $84.0 \pm 10.0 \text{ mg kg}^{-1}$ (Mn), 0.155 ± 0.012 to $0.300 \pm 0.009\%$ (Mg), 0.033 ± 0.004 to $0.667 \pm 0.048\%$ (Na), 1.01 ± 0.10 to $1.66 \pm 0.08\%$ (P), 0.134 ± 0.022 to $0.573 \pm 0.016\%$ (S), 49.0 ± 4.3 to $316 \pm 9 \text{ mg kg}^{-1}$ (Zn), < 0.03 to $4.82 \pm 0.74 \text{ mg kg}^{-1}$ (As), 1.85 ± 0.50 to $21.0 \pm 2.4 \text{ mg kg}^{-1}$ (Ba), < 0.02 to $0.102 \pm 0.012 \text{ mg kg}^{-1}$ (Cd), 0.083 ± 0.012 to $6.47 \pm 0.92 \text{ mg kg}^{-1}$ (Co), < 0.7 to $1.02 \pm 0.31 \text{ mg kg}^{-1}$ (Cr), 2.58 ± 0.82 to $21.0 \pm 0.88 \text{ mg kg}^{-1}$ (Cu), 0.430 ± 0.033 to $1.09 \pm 0.17 \text{ mg kg}^{-1}$ (Ni), 0.182 ± 0.043 to $0.442 \pm 0.088 \text{ mg kg}^{-1}$ (Pb), 0.038 ± 0.013 to $0.051 \pm 0.003 \text{ mg kg}^{-1}$ (Sb), 0.088 ± 0.001 to $1.91 \pm 0.08 \text{ mg kg}^{-1}$ (Se), < 0.3 to $23.0 \pm 0.9 \text{ mg kg}^{-1}$ (Sr), and 0.075 ± 0.004 to $0.665 \pm 0.075 \text{ mg kg}^{-1}$ (V), and for all samples B concentrations were $< 3.3 \text{ mg kg}^{-1}$. The complete information can be seen in Tables S3 and S4, in Supplementary material.

The elemental concentrations found in feed samples for fish for human consumption were in the following descending order: P > Ca > K > Na > S > Mg > Al > Fe > Zn > Mn > Sr > Ba > Cu > Co > As > Se > Cr > Ni > V > Pb > Cd > Sb > B (Table 5). The mean concentration for P (1.31%) was in agreement with the AAFCO guidelines, which establishes a minimum limit of 0.6% for this element (AAFCO, 2003).

The manufacturers did not report the minimum values present for elements not required by legislation, only for Ca and P, whose requirement is the information of the minimum and maximum values for Ca and the minimum for P. Mean concentrations found for Ca (1.30%) were within the reported range of the product labels, which expressed a minimum mean concentration of 0.97% and maximum of 1.51%. On the other hand, the mean minimum concentration for P reported on product labels was 0.93% and it was found 1.36%, an amount 1.46 times higher than the one reported by manufacturers.

It is noteworthy that among the elements determined in fish feed samples are included those with an important role in biological processes, such as P, that is an essential component of nucleic acids and cell membranes and its deficiency causes low growth, food inefficiency, and bone mineralization. In species such as *Puntius tetrazona* and *Poecilia reticulata*, the daily percentages range from 0.52 to 1.52% of the initial weight to achieve a corporeal gain (NRC, 1993; Zuanon, et al., 2011). In this work, concentrations ranging from 0.36 to 2.79% in ornamental fish feed samples and between 1.01 and 1.66% in feed for human consumption fish were found, which are in agreement with the range of the required levels.

Magnesium is an essential cofactor for many enzymatic reactions, in the intermediate metabolism, and plays an important role in respiratory adaptation of freshwater fish. Quantitative dietary Mg requirements in rainbow trout, carp, and catfish have been calculated and vary between 0.04 and 0.06% of the diet (NRC, 1993; Zuanon, et al., 2011). In our

Table 5
Statistical summary of the concentrations of inorganic constituents in feeds for fish intended for human consumption.

| Element | Mean | Median | Minimum | Maximum | n |
|---------------------------|-------|--------|---------|---------|---|
| Al (%) | 0.024 | 0.024 | 0.011 | 0.036 | 2 |
| Ca (%) | 1.30 | 1.21 | 0.787 | 2.00 | 4 |
| Fe (mg kg ⁻¹) | 269 | 137 | 128 | 542 | 3 |
| K (%) | 0.781 | 0.783 | 0.681 | 0.876 | 4 |
| Mg (%) | 0.204 | 0.181 | 0.155 | 0.300 | 4 |
| Mn (mg kg ⁻¹) | 46.0 | 42.0 | 17.0 | 84.0 | 4 |
| Na (%) | 0.456 | 0.562 | 0.033 | 0.667 | 4 |
| P (%) | 1.36 | 1.39 | 1.01 | 1.66 | 4 |
| S (%) | 0.393 | 0.433 | 0.134 | 0.573 | 4 |
| Zn (mg kg ⁻¹) | 122 | 61 | 49 | 316 | 4 |
| As (mg kg ⁻¹) | 2.81 | 2.72 | 0.902 | 4.82 | 3 |
| Ba (mg kg ⁻¹) | 8.95 | 6.47 | 1.85 | 21 | 4 |
| Cd (mg kg ⁻¹) | 0.082 | 0.083 | 0.060 | 0.102 | 3 |
| Co (mg kg ⁻¹) | 4.08 | 4.88 | 0.083 | 6.47 | 4 |
| Cr (mg kg ⁻¹) | 0.758 | 0.755 | 0.498 | 1.02 | 3 |
| Cu (mg kg ⁻¹) | 8.76 | 5.72 | 2.58 | 21 | 4 |
| Ni (mg kg ⁻¹) | 0.613 | 0.465 | 0.430 | 1.09 | 4 |
| Pb (mg kg ⁻¹) | 0.319 | 0.327 | 0.182 | 0.442 | 4 |
| Sb (mg kg ⁻¹) | 0.042 | 0.040 | 0.038 | 0.051 | 4 |
| Se (mg kg ⁻¹) | 0.917 | 0.834 | 0.088 | 1.91 | 4 |
| Sr (mg kg ⁻¹) | 17.0 | 21.0 | 7.92 | 23 | 3 |
| V (mg kg ⁻¹) | 0.394 | 0.418 | 0.075 | 0.665 | 4 |

n = number of fish feed samples using statistical summary.

work, the Mg concentration found varied from 0.15 to 0.29% in ornamental fish feed and 0.16 to 0.30% in feed for fish for human consumption, and are higher than the recommended levels.

Iron is an essential element in cellular respiratory process. But there are side effects of excessive up taking, whose main consequences include the poor growth, increase of mortality, diarrhea and histopathological damage to liver cells. The need for daily iron varies according to species but ranges from 0.30 to 170 mg kg⁻¹ (NRC, 1993; Zuanon, et al., 2011). In our study it was detected iron levels of 136 to 954 mg kg⁻¹ in ornamental fish feed and between < 18 and 542 mg kg⁻¹ in feed for fish for human consumption. These data suggest that there is much variability in Fe concentration in fish feeds and that it can be below the estimated necessary concentration for some species.

Copper is a component of many enzymes and is essential for many physiological activities. The content of Cu required in the diet of some species of fish is estimated to range between 3.0 and 5.0 mg kg⁻¹ (NRC, 1993). In our work, in some feed samples concentrations up to 9-times higher than those values were found, from 4.40 to 45.0 mg kg⁻¹ in ornamental fish feed and between 2.58 and 21.0 mg kg⁻¹ in feed for fish for human consumption.

The concentrations of elements found in this work for fish feed samples, are in concordance with the Brazilian (MAPA) and American (AAFCO) legislation, as well as with values estimated by literature reported for this type of samples (AAFCO, 2003; Brazil, 2009e; NRC, 1993; Zuanon, et al., 2011).

4. Conclusion

A 2⁵⁻² factorial and Doehlert designs, applied to optimize the sample decomposition and operational conditions of inductively coupled plasma optical emission spectrometry, were effective chemometric tools employed for analysis of dry fish feed samples, carried out in this work.

The sample preparation promoted a low residual acidity (~1.0 mol L⁻¹) in the final solutions, which could be directly used for analysis by ICP OES and, after a simple dilution, for analysis by ICP-MS. The ICP-based analytical methods were accurate and precise for the determination of macroelements (Ca, K, Na, Mg, P, and S), microelements (Cu, Fe, Mn, and Zn) and trace elements (Al, As, Ba, Cd, Co, Cr, Ni, Pb, Sb, Se, Sr and V) contained in fish feed (ornamental and for

human consumption), as revealed by the agreement obtained for certified reference materials.

Ornamental fish feed presented higher levels of Al, B, Ca, Fe, K, Mg, Mn, Na, S, Zn, V, Cr, Co, Ni, Cu, Se, Sr, Cd, Sb, Ba, and Pb and lower levels of P and As than feeds for fish for human consumption.

Contents of Cu, Fe, Mn, Se, and Zn in dry feed for ornamental fish were higher than the values reported by the manufacturers, whereas the Ca content was below the minimum value reported in the labels. Concentration of P was 1.62-times higher the minimum reported by manufacturers.

Samples of dry feeds for fish for human consumption contain Ca concentration within the minimum and maximum values reported by manufacturers, whereas the P concentration was 1.46-times higher than the minimum value reported on the product labels. Both types of fish feed (for ornamental and for human consumption purposes) contain P concentrations below the maximum allowed by the AAFCO (0.6%).

In this work, the elemental concentrations obtained for the dry fish feed samples evaluated were in accordance with the Brazilian and American legislation for the regulated elements. However, it is important the inclusion of more elements, especially toxic elements such as As, Cd, Hg, Pb, and others, to the list of those controlled by legislation, in order to ensure greater safety.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2018.01.178>.

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