ORIGINAL ARTICLE

Iron species and proteins distribution in unconventional food plants

Distribuição de espécies de ferro e proteínas em plantas alimentícias não convencionais

Aline Pereira de Oliveira[1], Juliana Naozuka[1]*

[1]Universidade Federal de São Paulo (UNIFESP), Departamento de Química, Diadema/SP - Brasil

*Corresponding Author: Juliana Naozuka, Universidade Federal de São Paulo (UNIFESP), Departamento de Química, R. Prof. Artur Riedel, 275, CEP: 09972-270, Diadema/SP - Brasil, e-mail: j.naozuka@unifesp.br

Abstract

The sustainable food valorization is capable to stimulate the local products consumption with quality and nutritional security. In this scenario, Unconventional Food Plants (UFPs) deserve attention representing an alternative for rural communities and contributing to the local and regional economy. This work aimed to add nutritional information, once it evaluates total Fe concentration, Fe-species (water soluble, acid soluble, and inorganic), proteins, and Fe- proteins distribution in beldroega (*Portulaca oleracea* L.), guasca (*Galinsoga parviflora* Cav.), ora-pro-nóbis (*Pereskia aculeata* Mill.), piracá (*Vernonia scorpioides* (Lam.) Pers.), and trapoeraba (*Commelina benghalensis* L.). Considering the Fe total concentration, the studied UPFs can be excellent Fe sources, when compared to foods of plant origin widely consumed and cultivated, detaching the guasca leaves (687 ± 19 μg g^{-1}). However, the guasca leaves showed low concentration of Fe associated to macromolecules and Fe soluble species (1.6 ± 0.3 μg g^{-1}). These results may justify the low bioavailability of Fe species. On the other hand, beldroega leaves presented the highest concentration of inorganic Fe (1.3 ± 0.2 μg g^{-1}). In the UFPs, there is high glutelins concentration when compared to the other protein groups, meaning that UFPs can be low-cost alternative to supplementing protein intake. Finally, for majority UFPs, except guasca leaves, Fe is mainly associated to albumins, being a good source of bioavailable Fe species.

Keywords: Unconventional food plants; Speciation; Inorganic iron; Soluble iron; Proteins; Fe-proteins.

Resumo

A valorização de alimentos sustentáveis é capaz de estimular o consumo de produtos locais com qualidade e segurança nutricional. Nesse cenário, as plantas alimentícias não convencionais (PANCs) merecem destaque, representando uma alternativa para as comunidades rurais e contribuindo para a economia local e regional. Este trabalho visa agregar informações nutricionais através da avaliação da concentração total de Fe, espécies de Fe (solúvel em água, solúvel em ácido e inorgânico) e proteínas, e da distribuição de Fe associado às proteínas em beldroega (*Portulaca oleracea* L.), guasca (*Galinsoga parviflora*), ora-pro-nóbis (*Pereskia aculeata*), piracá (*Vernonia scorpioides*) e trapoeraba (*Commelina benghalensis* L.). Considerando-se a concentração total de Fe, as PANCs podem ser uma excelente fonte de Fe, quando comparadas aos alimentos de origem vegetal comumente consumidos e cultivados, destacando-se as folhas de guasca (687 ± 19 μg g^{-1}). Entretanto, as folhas de guasca mostraram baixa concentração de Fe associado a macromoléculas (como proteínas) e espécies solúveis em água

(1,6±0,3 µg g^{-1}). Esses resultados podem justificar a baixa biodisponibilidade das espécies de Fe. Por outro lado, as folhas de beldroega apresentaram a maior concentração de Fe inorgânico (1,3±0,2 µg g^{-1}). Nas PANCs, existe alta concentração de glutelínas, quando comparadas às concentrações dos outros grupos proteicos, significando que as PANCs podem ser uma alternativa de baixo custo para suplementar a ingestão de proteínas. Portanto, para a maioria das PANCs, exceto folhas de guasca, o Fe está principalmente associado às albuminas, sendo uma boa fonte de espécies de Fe biodisponível.

Palavras-chave: Plantas alimentícias não convencionais; Especiação; Ferro inorgânico; Ferro solúvel; Proteínas; Fe-proteínas.

1 Introduction

The current global agri-food system is supported by conventional agricultural matrix and also predominantly limited and industrialized food pattern, which contributes to the unfamiliarity and underutilization of native species with high economic and nutritional potential (Leal et al., 2018). The current rural system encourages the monocultures cultivation to meet the high demand for commonly consumed food, such as rice, beans, and wheat (Kelen et al., 2015; Leal et al., 2018). In addition, it is necessary to promote the consume the sustainable food that maximizes the consumption of local products with quality and nutritional security, respecting for biodiversity and adding value to farming, mainly family farming, and favoring a cultivation with low environmental impact.

It is estimated there are more than 10,000 plants potentially edible, highlighting Unconventional Food Plants (UFPs). The UFPs are exotic plants that, although edible, are not often used as food by most of the people, but may be part of the culture of some regions (Kinupp & Lorenzi, 2014; Liberato et al., 2019; Paschoal & Souza, 2015). These plants represent a subsistence alternative for rural communities and can contribute to the local and regional economy. The UFPs are often consumed as salad, braised, in forms of sweets, juices, or used for medicinal purposes (Luizza et al., 2013; Paschoal & Souza, 2015; Brasil, 2010).

In general, UFPs are pest-resistant plants which are able to be grown in different climate conditions and can occupy areas where there is little sunshine, few fertile soils, or extreme humidity conditions that do not favor the cultivation of conventional food plants in addition to dispensing with the use of fertilizers and agricultural pesticides (Kinupp & Lorenzi, 2014; Liberato et al., 2019; Paschoal & Souza, 2015). The cultivation of UFPs assist in harnessing previously unproductive areas as well as this cultivation can bring a greater supply of healthy and low-cost food throughout the year (Luizza et al., 2013).

Studies on these edible plants show potential health effects, especially in relation to antioxidant protection and anti-inflammatory effects (Anusuya et al., 2012; Matu & Van Staden, 2003; Pinto et al., 2012). The UFPs are often consumed as salad, braised, in forms of sweets, juices, or used for medicinal purposes (Brasil, 2010; Kinupp & Lorenzi, 2014; Luizza et al., 2013). Among the various UFPs, some leaves can be detached, such as (1) "beldroega" (*Portulaca oleracea* L.) which is used as edible plant and traditional herbal medicine besides its leaves may help reduce the occurrence of cancer and heart disease as well as having anti-oxidation, anti-virus, anti-ulcerogenic, and anti-inflammatory properties (Chen et al., 2003; Xu et al., 2006); (2) "guasca" (*Galinsoga parviflora* Cav.) is used as a vegetable, seasoning, accompanying rice, roots, or white meat in which its leaves are good sources of protein, calcium (Ca), and magnesium (Mg), additionally can be applied for medicinal purposes with anti-inflammatory and antibacterial activities (Damalas, 2008; Odhav et al., 2007); (3) "ora-pro-nóbis" (*Pereskia aculeata* Mill.) is one of the most known UFPs which contain high levels of proteins, minerals, dietary fiber, vitamin A, vitamin C, and folic acid, consumed as salad or braised accompanying savory fillings and beans (Almeida & Corrêa, 2012; Martinevski et al., 2013; Takeiti et al., 2009); (4) "trapoeraba" (*Commelina benghalensis* L.) is consumed as salad, braised, in forms of sweets, juices, or used for medicinal purposes and used as a popular medicine, due to antibacterial, sedative, and analgesic properties, i.e., this edible plant contains alkaloids, volatile oil, wax, vitamin C and high levels of vitamin A, and β-carotene (Anusuya et al., 2012).

Studies about elemental chemical composition are paramount, since there are elements that play an important role in nutritional and medicinal values of plants. In this way, an example that should be highlighted is the iron (Fe) concentration in spinach which contributes as the valuable sources of Fe, being present in the daily diet mainly of the low-income socioeconomic classes (Yadav & Sehgal, 2002). However, it is important to determine the Fe species present in food because in plants there is non-heme Fe, which is absorbed in smaller amounts when compared to heme Fe species from sources of animal origin. The presence of oxalates, phosphates, phytates, and tannins in plants results in the formation of insoluble complexes with non-heme Fe, limiting their absorption (Powell et al., 1999). Beans also have low concentration of bioavailable Fe species and are not able to supply nutritional needs in cases of deficiencies (Oliveira & Naozuka, 2015).

Iron deficiency is one of the major public health problems in developing countries, such as Brazil, reaching levels up to 70% of the population in some regions (Obelar, 2008). Considering the worldwide profile, it is estimated that approximately 40% of the population presents Fe deficiency or low levels of hemoglobin establishing a risk situation that includes maternal-infant group (infants, preschoolers, school children, pregnant women, and nursing mothers) (Obelar, 2008).

The unknown potential of several UFPs requires further studies since these plants are promising from a medicinal and nutritional point of view and may be good alternative of Fe sources for human nutrition, especially in lower income socioeconomic classes in developing countries. Beside this, the UFPs cultivation can become an important tool in the production systems establishment in nutritious bases, valorization of family agriculture and polyculture production systems with low environmental impact rescuing the local biodiversity and the values of a more sustainable food. Therefore, this study aimed to determine the Fe total concentration and to evaluate the distribution of Fe-species (water soluble, acid soluble, inorganic, and Fe-proteins) and proteins in different UFPs as following: beldroega; guasca; ora-pro-nóbis; piracá; and trapoeraba.

2 Materials and methods

2.1 Instrumentation

For quantification of Fe total content, a Flame Atomic Absorption Spectrometer (F AAS) (Model AAS Vario 6, Analytik Jena AG, Germany) equipped with a deuterium lamp for background correction, was used. Acetylene flow of 70 L h^{-1}, airflow of 430 L h^{-1} and an observation height of 6 mm were maintained.

After fractionation procedures, the Fe quantification was performed in a Graphite Furnace Atomic Absorption Spectrometer (GF AAS) (Model ZEEnit® 60, AnalytikjenaAG, Jena, Germany) equipped with a transversely heated graphite atomizer, a pyrolytically coated graphite tube and a transverse-Zeeman-effect background corrector. The spectrometer was operated using hollow cathode lamp, operating with wavelength, lamp current and slit-width equal to 248.3 mm, 4 mA and 0.8 nm, respectively. All measurements were based on integrated absorbance values. Argon 99.998% (v v^{-1}) (Air Liquide Brasil, Brazil) was used as a protective and purge gas. The heating program for the graphite tube atomizer is shown in Table 1.

Table 1. Heating program for Fe determination by GF AAS.

Steps	Temperature (°C)	Ramp (°C s^{-1})	Hold (s)	Argon flow (mL min^{-1})
Drying	100	10	15	1000
Drying	130	10	20	1000
Pyrolysis	1200	100	20	1000
Atomization	2300	2300	5	0
Cleaning	2500	1200	2	1000

An Ultrospec 2100 Pro spectrophotometer (Biochrom LTD, Cambridge, UK), equipped with a xenon lamp and a wavelength range of 190-900 nm, was used for protein determination at 595 nm.

The samples and Standard Reference Material (SRM) digestion was carried out by a diluted oxidant mixture in a closed-vessel microwave system (Multiwave 3000, Anton Paar, Austria), equipped with sixteen fluoropolymer vessels and a ceramic vessel jacket.

In the proteins sequential extraction procedures and Fe chemical speciation, the homogenization of the culture medium was accomplished using an orbital shaker (model 0225M, Quimis, Brazil). A centrifuge (5415 R model, Eppendorf, Germany) was used to phase separation and protein precipitation.

For samples preliminary preparation, the leaves were dried using an oven (515 model, FANEM, SP, Brazil).

2.2 Reagents and samples

A mass of 500 g of different UFPs were purchased in the markets of the Sao Paulo city, in Brazil. The beldroega, ora-pro-nóbis, piracá, and trapoeraba species were purchased in the grocery, whereas guasca leaves in health foods stores.

All solutions were prepared from analytical reagent grade chemicals, using high-purity deionized water obtained from a Milli-Q water purification system (Millipore, USA) as the solvent. Analytical grade 65% (v v^{-1}) HNO_3 distilled in a quartz sub-boiling still (Marconi, Brazil) and 30% (v v^{-1}) H_2O_2 (Merck, Germany) were used to sample digestion.

Titrisol standard solution of 1000 mg L^{-1} of Fe (Merck, Germany) was used to prepare the reference analytical solutions in 0.14 mol L^{-1} HNO_3. The linear range used were 0.25-1.50 mg L^{-1} and 10-100 µg L^{-1} for Fe determination by F AAS and GF AAS, respectively.

The Fe total determination accuracy was evaluated by analysis of certified reference materials of peach leaves (SRM 1547) from the National Institute of Standards and Technology (NIST).

For sequential extraction procedure, acetone, chloroform, ethanol, methanol, sodium chloride (NaCl), and sodium hydroxide (NaOH) (Merck, Germany) were used. For evaluation of the protein precipitation procedure, it was used protein standard, prepared by dissolving 4.0 mg of ovalbumin (BioAgency, Brazil) in 2.0 mL of deionized water, 100% (w v^{-1}) trichloroacetic acid (Sigma-Aldrich, USA), acetone (Merck, Germany), and NaOH (Merck, Germany).

The protein total concentration was obtained using Bradford's reagent (BioAgency, Brazil), which was diluted five times with deionized water before analysis, and ovalbumin stock solution (Merck, Germany).

Different precipitant reagents were prepared for Fe speciation: (1) 25 g of trichloroacetic acid (Merck, Germany) and 25 mL hydrochloride acid (Synth, Brazil); (2) 25 g of trichloroacetic acid (Merck, Germany), 25 g of hydroxylamine hydrochloride (Merck, Germany), and 25 mL of hydrochloride acid (Synth, Brazil). For both precipitant reagents, final volume of 250 mL was completed by deionized water.

2.3 Sample preparation

The raw UFPs leaves were washed with deionized water and dried in an oven at 60 °C until constant mass. They were ground using a pestle and mortar (decontaminated) after freezing the samples in liquid nitrogen. Then, all results are presented in the dry sample mass.

For Fe determination, different leaves (beldroega, guasca, ora-pro-nóbis, piracá, and trapoeraba) or SRM were submitted to acid digestion in a closed-vessel microwave system. Sample masses ranging from 150 to 250 mg were digested, using a dilute oxidant mixture (2 mL HNO_3 + 1 mL H_2O_2 + 3 mL H_2O) (Naozuka et al., 2011). The heating program consisted of three steps (temperature/°C, ramp/min, hold/min): (1) (140, 5, 1); (2) (180, 4, 5); and (3) (220, 4, 10). The system was cooled through forced ventilation for 20 min. The digestion procedure was carried out in triplicates for each sample. After digestion, the samples and

blank solutions were transferred to plastic flasks and their volumes were made up to 15 mL with deionized water.

Digested solutions were analyzed by F AAS and instrument calibration was performed using Fe analytical solutions ranging from 0.25 to 1.50 mg L^{-1} in 0.1% w v^{-1} HNO$_3$.

The SRM 1547 was submitted to acid digestion and analyzed by F AAS to check the accuracy. Besides this, addition and recovery test was also applied to verify the matrix interferences, adding 0.30 mg L^{-1} of Fe analytical solution to sample, after acid digestion in a microwave oven.

For proteins and Fe-proteins determination, the sequential extraction procedure has been described by Naozuka & Oliveira (2007). A mass of 100 mg of dried and grounded leaves was used to solid-liquid sequential extraction with different extractants: methanol/chloroform mixture (1.5 mL, 1:2 v v^{-1}); acetone (1.5 mL, 75% v v^{-1}) (Do et al., 2014); deionized water (1.0 mL); NaCl (1.0 mL, 1 mol L^{-1}); ethanol (1.0 mL, 70% v v^{-1}); and NaOH (1.0 mL, 1 mol L^{-1}). The methanol/chloroform was used to defat, the acetone was to remove phenols and the water, NaCl, ethanol and NaOH were used to produce four protein fractions: albumin; globulin; prolamin; and glutelin, respectively. The extractions were carried out using an orbital shaker at 350 rpm for 30 min. The solid phase separation was carried out by centrifugation at 4000 rpm for 10 minutes.

Subsequently, supernatants were submitted to precipitation procedure using the mixture trichloroacetic acid (TCA)/acetone (Fic et al., 2010; Koontz, 2014). Protein denaturation and concentration was performed by adding 100 µL of TCA (100% w v^{-1}) to 400 µL of different supernatants (water, NaCl, ethanol and NaOH). The mixtures were shaken in orbital shaker at 350 rpm for 30 min, followed by incubation for 1 h in an ice bath. After that, the samples were centrifuged at 12,000 rpm for 10 min at 4 °C. The precipitated proteins were washed once with ice-cold acetone (500 µL) to remove any residual coloration and TCA, shaken in orbital shaker at 350 for 30 min, and centrifuged at 12,000 rpm for 10 min at 4 °C. Finally, the proteins were resuspended in 0.5 mL of NaOH (0.1 mol L^{-1}). The protein precipitation efficiency was previously evaluated submitting 400 µL of ovalbumin solution (0.2 µg L^{-1}) to precipitation procedure.

Protein determination was performed by the Bradford method (Bradford, 1976), using 595 nm wavelength. Spectrophotometer calibration was performed using analytical reference solutions of 4, 6, 8, 10, 12, 16, and 20 µg of ovalbumin in 1.0 mL of Bradford reagent.

Iron determination was carried out by GF AAS in the supernatants (water, NaCl, ethanol, and NaOH). Instrument calibration was performed using Fe analytical solutions ranging from 10 to 100 µg L^{-1} in 0.1% v v^{-1} HNO$_3$. The chemical interferences were evaluated by addition and recovery test, adding an Fe solution (20 µg L^{-1}) to different supernatants.

For Fe speciation, the Fe soluble and inorganic species determination was done using an adapted procedure proposed by Oliveira et al. (2018). The different procedures were adopted and are shown in the Figure 1.

For this, 7.0 mL of deionized water was added to 400 mg of dried and ground leaves. This mixture was submitted to agitation for 5 min at room temperature and supernatant was separated by centrifugation at 4,000 rpm for 10 min. The supernatants were analyzed by GF AAS, obtaining the Fe concentration in the aqueous extract (Fe soluble and Fe associated to macromolecules).

After that, it was added 2.5 mL of the precipitant reagent from 1 to 5 mL of aqueous extract, promoting the macromolecules precipitation (Koontz, 2014). The mixture was heated in a thermostatic bath at 95 °C for 10 min, centrifuged at 4000 rpm for 10 min and the supernatant was analyzed by GF AAS, obtaining the concentration of Fe soluble not associated to macromolecules (Quinteros et al., 2001).
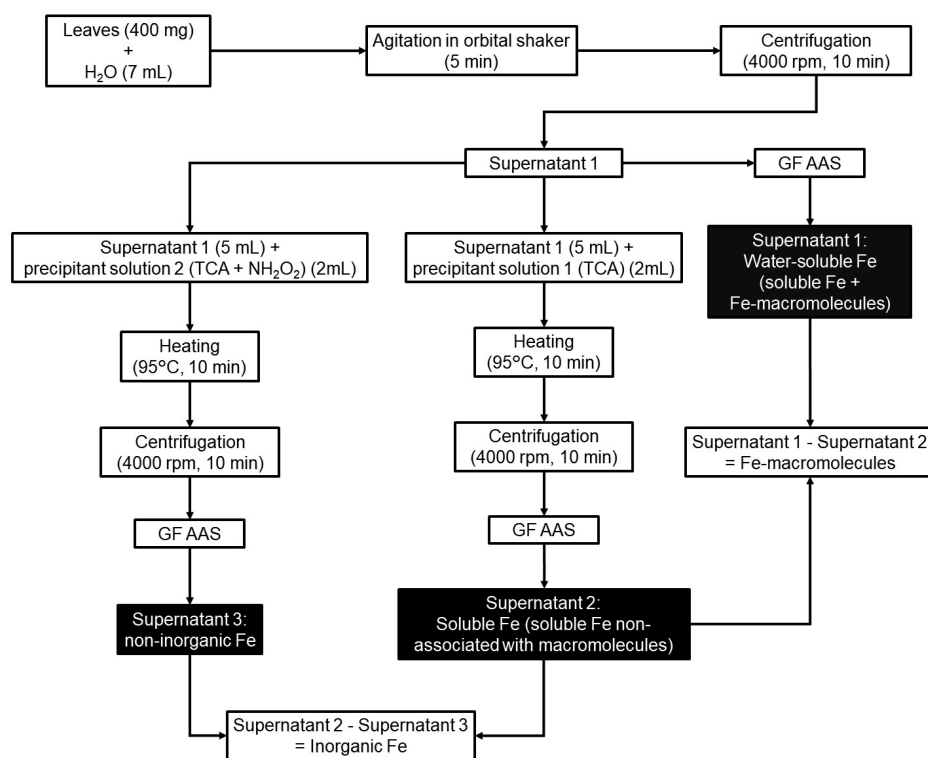
Figure 1. Flowchart of Fe speciation (Fe soluble, macromolecules and inorganic species).

Finally, 2.5 mL of the precipitant reagent 2 was added to 5 mL of the aqueous extract. The mixture was heated in a thermostatic bath at 95 °C for 10 min, centrifuged at 4000 rpm for 10 min and the supernatant was analyzed by GF AAS. This procedure was applied to precipitate macromolecules and promote the reduction of Fe(III) to Fe(II) (Quinteros et al., 2001).

The Fe determination in the supernatants was performed by GF AAS and the heating program according to Table 1. The volume of 10 μL of the samples or analytical solutions was injected into the graphite tube and no chemical modifier was used. The instrumental calibration was done with Fe analytical solutions ranging from 10 to 80 μg L^{-1} in 0.1% (v v^{-1}) HNO_3. To evaluate possible matrix interference in Fe determination, the addition and recovery test was performed. For this purpose, Fe analytical solution (20 μg L^{-1}) was added in the presence of the precipitant reagents 1 and 2, without and with addition of hydroxylamine, respectively.

3 Results and discussion

3.1 Characteristics method of Fe determination

The characteristics parameters for Fe determination by F AAS (total Fe) and GF AAS (Fe-proteins or Fe speciation), such as linear range, correlation coefficient (R^2), Limit of Detection (LOD) and Limit of Quantification (LOQ) are shown in Table 2. LOD was calculated using the standard deviation of 10 measurements of the analytical blank sample (LOD = 3 × σ_{blank}, where σ is the standard deviation), and LOQ = 3 × LOD. For total Fe, values were obtained in μg g^{-1} considering a sample mass of 150 mg and a final volume of 15 mL. For Fe-proteins, values were obtained in ng g^{-1}, considering a sample mass of 100 mg and a final volume of 1 mL. For Fe species, values were obtained in ng g^{-1} considering a sample mass of 400 mg and a final volume of 10 mL.

Table 2. Characteristics method for Fe determination by F AAS and GF AAS.

	Analytical Blank	Linear range	R^2	LOD	LOQ	Recovery (%)
F AAS*	HNO$_3$	0.25-1.50	0.9945	8.5	28.1	111
GF AAS**	Water	10-100	0.9984	20	61	113
	NaCl			37	111	96
	Ethanol			12	36	87
	NaOH			38	114	117
	Precipitant reagent 1	10-100	0.9984	20	60	108
	Precipitant reagent 2			60	190	103

F AAS: Flame Atomic Absorption Spectrometer. GF AAS: Graphite Furnace Atomic Absorption Spectrometer. LOD: Limit of Detection. LOQ: Limit of Quantification. *Linear range (mg L^{-1}). LOD and LOQ (μg g^{-1}). **Linear range (μg L^{-1}); LOD and LOQ (ng g^{-1}).

Recovery percentages, Table 2, ranged from 87 (ethanol) to 117 (NaOH)%. According to NBR ISO/IEC 17025, the recovery tolerance ranges from 80% to 120% (Burin et al., 2008), evidencing the matrix interferences absence in the Fe determination by GF AAS and F AAS. Additionally, the results quality obtained by F AAS was assessed by SRM 1547 analysis, which was subjected to the same experimental procedures adopted for the acid digestion of the samples. The results obtained showed that the analytical method is selective and accurate. The certified and experimental values were compared by Student's t test at 95% confidence limit and it was found that there was no significant difference, since the certified value is 219.8 μg g^{-1} and experimental Fe concentration was 217$\pm$17 μg g^{-1}.

3.2 Total Fe and Fe species (soluble and inorganic) in UFPs

The Fe total and species concentrations in UFPs (beldroega, guasca, ora-pro-nóbis, piracá, and trapoeraba) are shown in Table 3. The different Fe species were quantified in supernatants obtained after extraction with water, precipitant reagent 1 and precipitant reagent 2. After addition of the precipitant reagents 1 and 2, it was possible to quantify acid soluble non-macromolecular and inorganic Fe, respectively. The TCA addition could promote the proteins and peptides precipitation and hydroxylamine absence, then, it was possible to obtain the concentration of acid soluble Fe non-associated with macromolecules (Koontz, 2014; Oliveira & Naozuka, 2015). The TCA and hydroxylamine addition allowed the total inorganic Fe quantification, since Fe(III) was reduced to Fe(II) ions and Fe reduced species interactions with macromolecules, such as antinutritional components, forming solid compounds that suffer precipitation (Oliveira & Naozuka, 2015). Finally, it was possible to quantify Fe inorganic total, subtracting the Fe concentrations found in the supernatants obtained after addition of precipitant reagent 1 from precipitant reagent 2.

Table 3. Fe concentrations in supernatants and digested samples.

	Fe concentration (μg g^{-1}) $\pm$ Standard Deviation (n = 3)				
	Beldroega	Guasca	Ora-pro-nóbis	Piracá	Trapoeraba
Total	46 $\pm$ 2	687 $\pm$ 19	123 $\pm$ 6	283 $\pm$ 6	138 $\pm$ 2
Aqueous extract	2.5 $\pm$ 0.1[a]	1.6 $\pm$ 0.3[a]	5.7 $\pm$ 0.2[a]	5.7 $\pm$ 0.2[a]	3.8 $\pm$ 0.1[a]
Precipitant reagent 1 (without hydroxylamine, pH < 1)	2.4 $\pm$ 0.2[a]	1.0 $\pm$ 0.1[b]	4.6 $\pm$ 0.2[b]	4.6 $\pm$ 0.2[b]	3.3 $\pm$ 0.2[b]
Precipitant reagent 2 (with hydroxylamine, pH = 5,5)	1.1 $\pm$ 0.1[b]	0.55 $\pm$ 0.01[c]	5.0 $\pm$ 0.2[b]	5.0 $\pm$ 0.2[b]	2.9 $\pm$ 0.3[b]
Inorganic Fe*	1.3 $\pm$ 0.2	0.45 $\pm$ 0.10	ND**	ND**	ND**

*Subtraction of the Fe concentration in the extract with hydroxylamine of the extract without hydroxylamine. **ND: Not determined.
[a-c]Different superscripts within the same plant species indicate significant differences between Fe concentration in the supernatants ($p < 0.05$).

It was verified that guasca leaves is an excellent Fe source and an alternative for the anemia treatment caused by Fe deficiency, since it showed the highest Fe total concentration (687$\pm$19 μg g^{-1}). The results indicated that guasca leaves were characterized as higher Fe bioaccumulator than others studied plants. Considering the Dietary Reference Intake (DRI) of Fe for an adult male (31 to 50 years old) equal to 18 mg

(Dolan & Capar, 2002), the guasca consumption of 26.2 g of leaves would reach the Fe daily recommendation. For others UFPs, the same mass (26.2 g) would contribute between 6.7 (beldroega) and 41.2 (piracá)% of the Fe DRI.

Studies published previously by our research group indicated that the black bean showed the highest Fe total concentration (94 ± 3 ug g^{-1}) (Oliveira & Naozuka, 2015). However, the guasca and piracá leaves have Fe concentrations of seven and three times, respectively, higher than black beans, detaching the UFPs potential to supply Fe deficiencies. Besides that, it is important to point out that comparisons between obtained results and other literature works is difficult, since that genetic and physiology differences, soil characteristics, different water source and external factors (*eg*. fertilizers, insecticides, and pesticides) can vary the essential elemental concentrations in the UPFs (Harnly et al., 2009). An example of this is associated with the results obtained by Oliveira et al. (2013), where a high Fe concentration was found in the bedroega when compared to ora-pro-nóbis, being the opposite to the observed in this work (Fe concentrations in bedroega (46 ± 2 µg g^{-1}) and ora-pro-nóbis (123 ± 6 µg g^{-1}).

It is important to know the Fe species concentration, which are potentially absorbed by the human organism, since the total Fe concentration cannot provide essentiality and toxicity information (Naozuka, 2018). Non-heme Fe species can be found in vegetable foods, such as Fe(II) and/or Fe(III). However, less than 5% of these Fe species are absorbed by human organism, being that Fe(II) is more available than Fe(III) (Quinteros et al., 2001). It is possible that in the fraction containing Fe soluble and Fe associated to macromolecules (aqueous extract) there are Fe species available, mainly Fe associated to albumins. In relation to Fe total concentration, the Fe concentration in the aqueous extract ranged from 0.2% (guasca) to 5.4% (beldroega). It is interesting to observe that, even though guasca act as a good Fe source, this leave had the lowest concentration of Fe species potentially available (1.6 ± 0.3 µg g^{-1}).

The decrease in Fe concentration in the supernatant after addition of precipitant reagent 1, ranged from 0 (beldroega) to 20% (ora-pro-nóbis and piracá) when compared to the Fe concentration in the aqueous extract. It was possible to infer that in studied UFPs there is a small percentage of Fe soluble associated to macromolecules.

Comparing the Fe concentrations after the addition of the precipitant reagents 1 and 2, it was verified that the Fe concentration decreased only for the beldroega (54%) and guasca (45%) leaves. Although beldroega leaves had the lowest total Fe concentration, it was the plant species that presented the highest concentration of inorganic Fe (1.3 ± 0.2 µg g^{-1}). Lastly, although ora-pro-nóbis, piracá, and trapoeraba leaves presented the highest concentration of water-soluble Fe species, inorganic Fe species were not determined, indicating that water-soluble Fe species in these leaves are associated to compounds of low molecular weight and soluble fibers, which are not precipitated by the action of trichloroacetic acid and hydroxylamine.

3.3 Proteins and Fe-proteins distribution in UFPs

The sequential extraction using the extractants water, NaCl, ethanol, and NaOH allows the separation of albumin, globulin, prolamin and glutelins, respectively (Fabian & Ju, 2011). The protein quantification was done by Bradford method (Bradford, 1976) obtaining analytical signals in wavelength in the visible region (595 nm). Therefore, it was necessary the protein isolation/purification by precipitation, using a mixture of TCA and acetone, since an intense green color was observed in the supernatants obtained after sequential extraction, due to chlorophyll presence. This procedure was evaluated using standard solution of ovoalbumin in which 100% of recovery was achieved. Additionally, the LOD and LOQ were also obtained, considering 100 mg sample mass and a final volume of 0.5 mL, corresponding to 66 and 198 µg g^{-1}, respectively.

The concentrations of albumin, globulin, prolamin, glutelin and Fe associated to these protein types are presented in Table 4. The glutelin group is in higher concentrations for most of the studied leaves, ranging from 0.20 ± 0.02 (guasca) to 3.1 ± 0.3 (piracá) mg g^{-1}. On the other hand, prolamin group showed the lowest concentrations in all leaves, all of them below the LOQ. The evaluation of the amino acids bioavailability in

the determined protein group is an important role for proteins nutritional value in plants. Studies by Chan & Phillips (1994) in plant origin foods indicated that the prolamins and glutelins have the highest concentrations of methionine, which is a sulfur-rich amino acid. Therefore, higher glutelins concentration than the other protein groups in the evaluated UFPs may indicate a high nutritional value of these foods. In addition, considering the protein concentration sum of the different groups, piracá leaves had the highest proteins concentration (5.5 ± 0.3 mg g^{-1}), being similar to those found in bean sprouts published in previous studies of our research group (Oliveira et al., 2013, 2017, 2018). In summary, UFPs may be a low-cost alternative to supplementing protein intake, mainly in developing countries with protein malnutrition.

Metalloproteins, such as Fe-proteins, in foods are chemical species potentially absorbed by human body. The Fe metalloproteins formation can be explain due to presence of amino acids, as methionine, cysteine, glutamic acid, arginine, aspartic acid, and lysine, that are rich in sulfur and charged groups, which have high affinity toward transition metal ions and non-metals (Garcia et al., 2006). In general, it was found that Fe is mainly associated to proteins from the albumins, Table 4, ranging from 1.1 ± 0.1 (guasca) to 13.7 ± 0.3 (piracá) µg g^{-1}, and less associated to prolamins group proteins, ranging from 0.26 ± 0.01 (ora-pro-nóbis) to 0.96 ± 0.04 (trapoeraba) µg g^{-1}. Only the guasca leaves showed that Fe was mainly associated to prolamins. Comparing the sum of the concentration of Fe associated to proteins with Fe total concentration, it was verified that it ranged from 1.2 (guasca) to 10.5% (ora-pro-nóbis). Although ora-pro-nóbis leaves presented one of the lowest Fe total concentrations, these leaves had the highest Fe-proteins concentration.

Table 4. Protein and Fe-protein concentrations in different UFPs leaves.

	Protein concentration (mg g^{-1}) ± Standard Deviation (n=3)			
	Albumin	**Globulin**	**Prolamin**	**Glutelin**
Beldroega	0.29 ± 0.02	< LOQ	< LOQ	1.2 ± 0.1
Guasca	< LOQ	< LOQ	< LOQ	0.20 ± 0.02
Ora-pro-nóbis	2.0 ± 0.1	0.8 ± 0.1	< LOQ	0.75 ± 0.04
Piracá	1.8 ± 0.1	0.62 ± 0.02	< LOQ	3.1 ± 0.3
Trapoeraba	< LOQ	< LOQ	< LOQ	0.65 ± 0.01
	Fe concentration (µg g^{-1}) ± Standard Deviation (n=3)			
Beldroega	1.2 ± 0.2	0.54 ± 0.01	0.42 ± 0.04	0.9 ± 0.1
Guasca	1.1 ± 0.1	0.24 ± 0.01	4.4 ± 0.3	2.3 ± 0.1
Ora-pro-nóbis	8.9 ± 0.3	2.3 ± 0.1	0.26 ± 0.01	1.5 ± 0.2
Piracá	13.7 ± 0.3	1.1 ± 0.1	0.61 ± 0.01	6.1 ± 0.4
Trapoeraba	7.3 ± 0.1	1.7 ± 0.2	0.96 ± 0.04	2.4 ± 0.1

4 Conclusions

Considering the Fe total concentration, the studied UPFs (beldroega, guasca, ora-pro-nóbis, piracá, and trapoeraba) can be excellent Fe sources, when compared to foods of plant origin widely consumed and cultivated. It is important to point out that Fe concentrations in the UPFs may change due to genetic and physiology differences, soil characteristics, different water source and external factors (*eg*. fertilizers, insecticides, and pesticides). The guasca leaves presented high Fe concentration, but low concentration of soluble Fe associated to macromolecules and inorganic Fe species. These results may justify the low bioavailability of Fe species.

In the UFPs, there is high glutelins concentration compared to the other protein groups, meaning that UFPs can be low-cost alternative to supplementing protein intake. Finally, for majority UFPs, except guasca leaves, Fe is mainly associated to albumins, being a good source of bioavailable Fe specie.

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