

# Optimization and Validation of a Simple Method for Mineral Potential Evaluation in Citrus Residue

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**Abstract** The aim of this study was to optimize and validate a simple method for determination of the potential of important minerals for health (calcium, iron, zinc, and magnesium) in residue from orange and lemon juice industries and determine whether there are differences concerning these nutrients during the months when these fruits were harvested. The mineralization was optimized using an experimental design and then validated. Dried residue samples of Pera orange, Hamlin orange, Tahiti lime, Sicilian lemon, and a mixture composed of oranges and lemons were digested and analyzed by flame atomic absorption spectrophotometry (FAAS). Citrus fruit samples, harvested in different months, were also analyzed. The most appropriate condition for mineralization was the use of the highest values of sample mass (0.6 g) and nitric acid volume (8.0 mL). All the parameters of validation were met. The average mineral content levels found were 6.8 mg/g for calcium and 116.4, 915.5, and 7.4 µg/g for iron, magnesium, and zinc, respectively. Thus, a portion of 100 g of residue can provide 68.3% of the Recommended Daily Intake (RDI) for calcium, 35.2% for magnesium, 83.1% for iron, and 10.6% for zinc. The principal component analysis showed no clear separation among oranges, lemons, and the mixture as for their composition. The mineral content levels found indicate that citrus residue has nutritional potential for use in human food and can contribute significantly to the achievement of the RDIs, especially for calcium and iron, since their deficiencies are considered major public health problems.

**Keywords** Iron · Calcium · Zinc · Magnesium · Experimental design · Lime

## Introduction

The production of citrus fruits (orange/lemon) is one of the most important sectors of Brazilian agribusiness, with annual harvest of approximately 18 million tons of orange, which corresponds to 30% of the world production; 80% of the Brazilian production is intended for the juice industry (IBGE 2014; MAPA 2015). The lemon production is over 1 million tons, and the state of São Paulo accounts for most of the citrus produced in the country (IBGE 2014).

In addition to juice, essential oils from peel, aromatic liquid, bran, and residue are generated from orange and lemon processing. Citrus residue is used in the composition of animal feed (Munhoz and Morabito 2010); it may also be used as coal for generation of energy (Rezzadori and Benedetti 2009). Some studies assess the use of orange and lemon residue as biomass for production of biogas, bioethanol, and bioenergy as a way to reduce the environmental impact caused by the disposal of such residue on the environment (Bożym et al. 2015; Choi et al. 2015; Vamvuka et al. 2014). Citrus residue has been used in fermentation processes for the production of enzymes, for the degradation of substances (Júnior et al. 2012), and for obtaining bioactive phenolics (Júnior et al. 2014). The fibers of some citrus were studied as potential functional ingredients in foods like ice cream, cookies, and cereal bar, undergoing evaluation as for physicochemical and technological properties (López-Marcos et al. 2015; Santos et al. 2011).

The composition of citrus peel may vary depending on cultivar, fruit ripeness, climate, type of soil, and fertilization. Barros et al. (2012) reported that Pera orange and Tahiti lime

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peels produced in Brazil have calcium (Ca), iron (Fe), zinc (Zn), potassium (K), manganese (Mn), sodium (Na), magnesium (Mg), and copper (Cu) in their composition.

Minerals are essential for the body functioning, and the minerals Ca, Fe, Mg, and Zn participate in important biological functions; their deficiencies are related with the appearance of some diseases, such as osteoporosis, atherosclerosis, diabetes mellitus, and anemia (Etcheverry et al. 2012; Hambidge 2000; Lieu et al. 2001; Nielsen 2010; Serefko et al. 2013). According to the World Health Organization (WHO 2006), iron deficiency (e.g., anemia) is the most predominant in the world, reaching billions of people, constituting a serious public health problem (INACG et al. 1998).

Among the main analytical techniques for determination of minerals in foods, there is the flame atomic absorption spectrometry (FAAS) and the inductively coupled plasma atomic emission spectrometry (ICP OES), in addition to the visible spectrophotometry and other analytical techniques (Fraige et al. 2007; Skoog et al. 1998). Among them, flame atomic absorption spectrometry is the most widespread due to its low cost, simplicity, and few interfering factors (Dean and Ma 2008).

Sample preparation is one of the most important steps of mineral analysis by atomic spectrometry. In this step, the sample is added with nitric acid and hydrogen peroxide, or other strong acids, for degradation of organic matter, a process also known as mineralization (Alonso et al. 2015).

The experimental design enables the statistical evaluation as for the influence of variables and their interactions in a given process, with fewer assays compared to univariate optimization. Therefore, shorter time of analysis and lower cost in conjunction with response surface analysis enable the evaluation of the best working conditions (Neto et al. 2010; Rodrigues and Iemma 2009). The experimental design has rarely been used for the optimization of simultaneous determination of minerals by atomic absorption spectrometry (Pereira-Filho et al. 2002; Santos et al. 2008), highlighting its application in the sample preparation step (Trindade 2009). After optimization of the analytical procedures, validation is carried out to ensure that a method is reliable (BRASIL 2011).

Studies on mineral potential utilizing orange and lemon juice industrial residue are still scarce. Thus, this original research aimed at optimizing and validating an analytical method to evaluate the content levels of iron, calcium, magnesium, and zinc by flame atomic absorption spectrometry in samples consisting of orange and lemon residue and a mixture of oranges and lemons. Besides investigating the composition of these nutrients in different varieties of orange and lemon, the behavior of these minerals during the harvest period of the evaluated cultivars was determined.

## Material and Methods

### Material, Equipment, and Reagents

The samples were ground with a grinder (model A11, Ika) and weighed on an analytical balance (AP210-0 model, Ohaus) to perform the analyses. Glass tubes and a digester block (M242, Quimis model) were used for the mineralization of samples (110/130 °C), then the samples were solubilized in ultrasonic bath (model 1510, Branson). After measuring the volume in volumetric flasks, the digest was stored in Falcon polypropylene tubes (Sarstedt). All glassware used was washed in detergent bath (immersion minimum of 6 h) and rinsed with water. Then, they were immersed in a bath of nitric acid 10% (v/v) for at least 12 h. After this time, the glassware was rinsed with ultrapure water and dried at room temperature. All glassware used was calibrated.

For the analyses of calcium, iron, magnesium, and zinc, we used a flame atomic absorption spectrometer (FAAS), model AAnalyst 200, with a deuterium lamp for correction of background radiation and hollow cathode lamps for determination of iron (248.3 nm), calcium (422.67 nm), magnesium (285.21 nm), and zinc (213.86 nm) (PerkinElmer). Each sample was placed into the nebulizer and mixed with air-acetylene flame (2.5/10 L h<sup>-1</sup>) at approximately 2000 °C.

For the determination of residual carbon, we used an inductively coupled plasma atomic emission spectrometer (ICP OES), model Optima 2000DV (PerkinElmer). The mineralization efficiency was evaluated, according to the method proposed by Gouveia et al. (2001) using glucose as standard in concentrations of 0.1 to 1.5%.

We used nitric acid (Sigma-Aldrich) and hydrogen peroxide (Synth) for the mineralization of samples. For the construction of analytical curves, we used standard solutions of iron, calcium, magnesium, and zinc (Sigma-Aldrich), with concentration of 1000 mg g<sup>-1</sup>. The solutions were prepared in ultrapure water from the Sartorius system, model arium comfort (USA), and filtered in ash-free quantitative filter paper (Nalgon, 9 cm diameter).

### Samples

Dry residue samples of two oranges (*Citrus sinensis* cv. Pera and cv. Hamlin), Sicilian lemon (*Citrus limon*), Tahiti lime (*Citrus aurantifolia*), and mixtures (consisting of Pera orange, Hamlin orange, and Tahiti lime) of these citrus were donated by a local industry. The provided residue consisted of citrus peels (albedo and flavedo) and seeds, collected over a period from 3 to 6 months (during the harvest), totaling 21 samples. The essential oil had already been removed. Approximately 100 g of each sample was milled and homogenized for the analyses. For optimization and validation of the method, we

used a mixture composed of Pera orange, Hamlin orange, and Tahiti lime.

### Method for Mineral Analysis in Citrus Residue

#### Optimization of the Mineralization Step

The sample of citrus residue mixture used in this step of the study was mineralized based on the method proposed by Boen and Pallone (2009). Samples were weighed, and nitric acid was added, according to the proportions determined in the experimental design, and left to stand for one night. Then, 2 mL of hydrogen peroxide (30%) was added and mineralized in a digester block for 2 h at 110 °C and then for 2 h at 130 °C. After digestion and cooling, the samples were solubilized in an ultrasound bath and filtered with paper ash-free filter (Nalgon, 9 cm diameter). The digest was transferred to a volumetric flask and the volume measured up to 25 mL with ultrapure water. Through the analysis, the minerals calcium (Ca), iron (Fe), magnesium (Mg), and zinc (Zn) were evaluated. For the analysis of calcium and magnesium, lanthanum was added up to the concentration of 1% *w/v*. The samples were analyzed and quantified by flame spectrometer atomic absorption (FAAS).

In order to determine the sample mass and nitric acid volume which would provide efficient sample digestion, adequate recovery, and repeatability in the analysis, optimization of the mineralization process was carried out using full-factorial experimental design, composed of  $2^2$  (4 experiments) in duplicate and 3 central points, totaling 11 assays. For the optimization, the responses in concentration for iron, calcium, magnesium, and zinc were evaluated. The levels of the variables and the values assigned to them are presented in Table 1. The matrix of the assays can be observed in Table 2.

#### Validation of the Method for Determination of Iron, Calcium, Magnesium, and Zinc by FAAS

The method was validated in accordance with recommendations from the guidelines on validation of INMETRO and AOAC (AOAC 2002; BRASIL 2011). According to INMETRO, the parameters of sensitivity, linearity, limit of detection and quantification, accuracy, and recovery are used in the validation (BRASIL 2011).

For the evaluation of linearity and selectivity, analytical curves were constructed for each element analyzed, in six or seven concentration levels, by external standardization, in independent triplicates. Linearity was determined through the graph of assay results depending on the concentration, calculating the equation of linear regression using the least squares method, analysis of variance (ANOVA) of the regression, the residues, and the linear correlation coefficient (*r*). Sensitivity was expressed as the angular coefficient of the analytic curve.

**Table 1** Variables and levels optimized in experimental design

Variables	Levels			
	Code	-1	0	+1
Sample mass (g)	$x_1$	0.3	0.45	0.6
Nitric acid volume (mL)	$x_2$	6	7	8

The absence of outliers in the residues was determined by Jackknife test and the homogeneity variance by Levene test (BRASIL 2011).

The limit of detection (LOD) and limit of quantification (LOQ) were evaluated by reading the response of 10 blank samples and calculated as proposed in INMETRO (BRASIL 2011). Recovery and precision (determined by the parameters of repeatability and intermediate precision) were determined as suggested by the INMETRO guide (BRASIL 2011). The coefficient of variation (CV) values obtained were compared with the calculated CV as suggested by AOAC (2002).

#### Statistical Treatment

Samples of oranges, lemons, and mixture collected in different months of 2015 harvests were evaluated by analysis of variance (ANOVA) and Tukey test, with 95% confidence, using an extension of Microsoft Office Excel (version 2013) and Statistica 8.0 (StatSoft, Inc., Tulsa, USA). In order to obtain more information about the data, we used principal component analysis (PCA) using the PLS Toolbox v. 5.8 (Eigenvector, Research, Inc., USA) which is used in the Matlab 7.8 (MathWorks, USA).

## Results and Discussion

### Experimental Design to Determine Minerals in Citrus Residue

Table 3 presents the values obtained in the experimental design for the minerals Ca, Fe, Zn, and Mg.

**Table 2** Matrix of assays of the  $2^2$  experimental design with central point

Trials	Sample mass (g)	Nitric acid volume (mL)	Sample mass (g)	Nitric acid volume (mL)
1	-1	-1	0.3	6
2	+1	-1	0.6	6
3	-1	+1	0.3	8
4	+1	+1	0.6	8
5	0	0	0.45	7

**Table 3** Responses for the concentration of minerals obtained in the 2<sup>2</sup> factorial design with central point

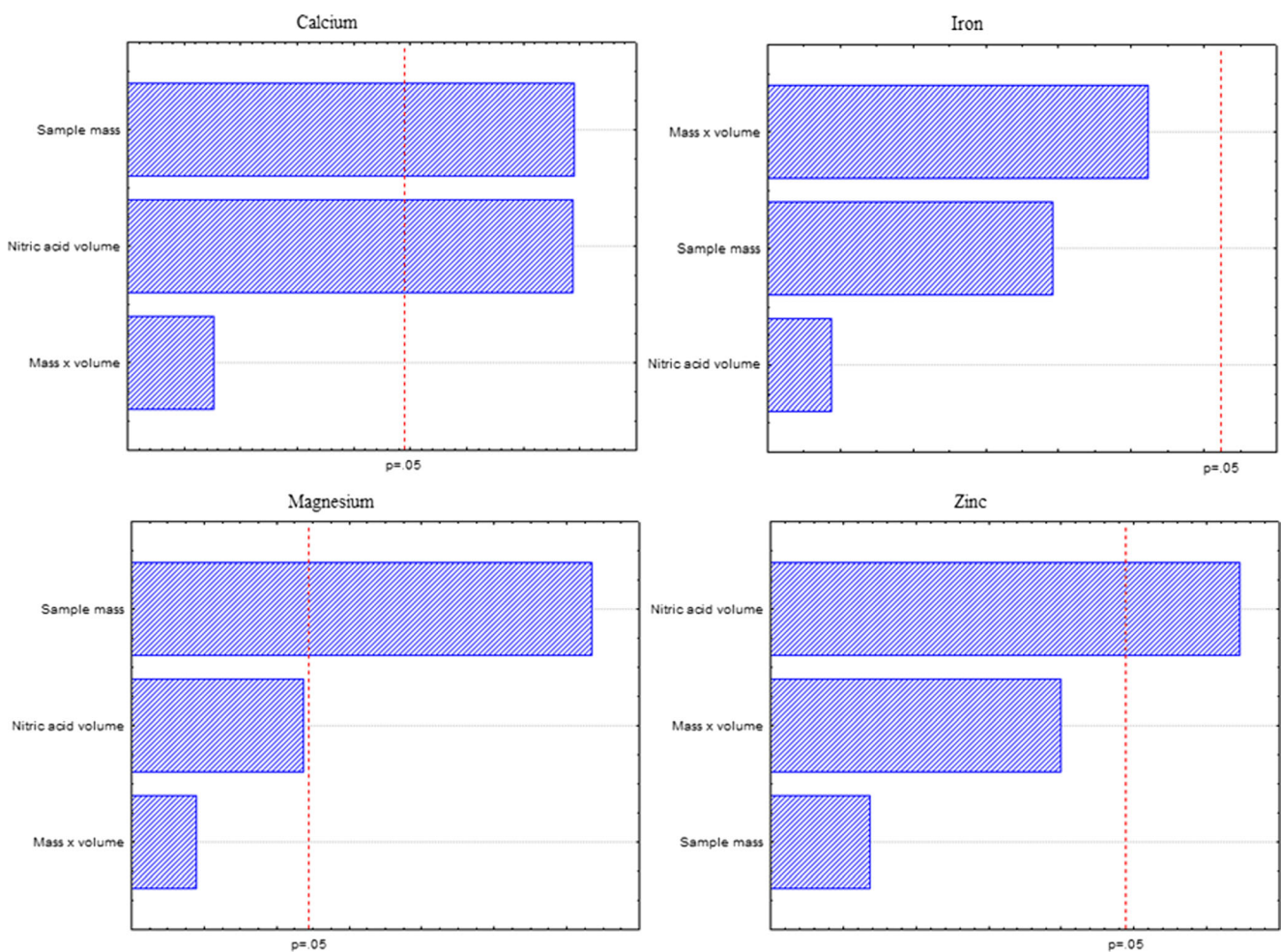
Trials	Concentration									Magnesium ( $\mu\text{g g}^{-1}$ )		
	Calcium ( $\text{mg g}^{-1}$ )			Iron ( $\mu\text{g g}^{-1}$ )		Zinc ( $\mu\text{g g}^{-1}$ )						
1	6.5	6.3		38.9	39.4	7.4	10.7			945.6	970.7	
2	6.6	6.7		37.5	41.2	9.0	6.6			899.8	898.3	
3	6.7	6.7		39.9	39.8	4.3	3.9			1008.0	979.4	
4	6.9	7.2		36.6	36.2	6.5	6.8			903.0	927.0	
5	6.7	6.8	6.9	36.7	35.4	35.8	6.5	7.1	8.5	947.8	936.7	964.8

The mean value for calcium content in the sample of mixture of citrus residue was  $6.8 \text{ mg g}^{-1}$ . For iron and zinc concentrations, it ranged from  $35.4$  to  $41.2 \mu\text{g g}^{-1}$  and from  $3.9$  to  $10.7 \mu\text{g g}^{-1}$ , respectively; for magnesium, the mean concentration found was  $943.7 \mu\text{g g}^{-1}$ .

Figure 1 presents the Pareto charts, where the effects of variables for each mineral are represented. The significant variables intersect the dashed line of  $p = 0.05$ .

According to Fig. 1, the variables, mass sample, and nitric acid volume have significant effect on sample mineralization

for calcium determination; however, the interaction effect between those variables was not significant. The increases in sample mass and nitric acid volume increased calcium concentration by  $0.3$  and  $0.3 \text{ mg g}^{-1}$ , respectively. Thus, to improve its mineralization, the most appropriate condition is the use of the highest sample mass ( $0.6 \text{ g}$ ) and the highest nitric acid volume ( $8 \text{ mL}$ ). The analysis of variance for calcium concentration demonstrated that there was no lack of fit for the model, which means that the data adjusted to a linear equation. The explained variance ( $R^2 = 82\%$ ) was also



**Fig. 1** Pareto charts representing the effects for calcium, iron, zinc, and magnesium



satisfactory. Moreover, it was observed that the factors of sample mass and nitric acid volume are highly significant, since the  $p$  value is lower than 0.05. Thus, it was possible to get a contour curve for calcium concentration as a function of sample mass and nitric acid volume (Fig. 2).

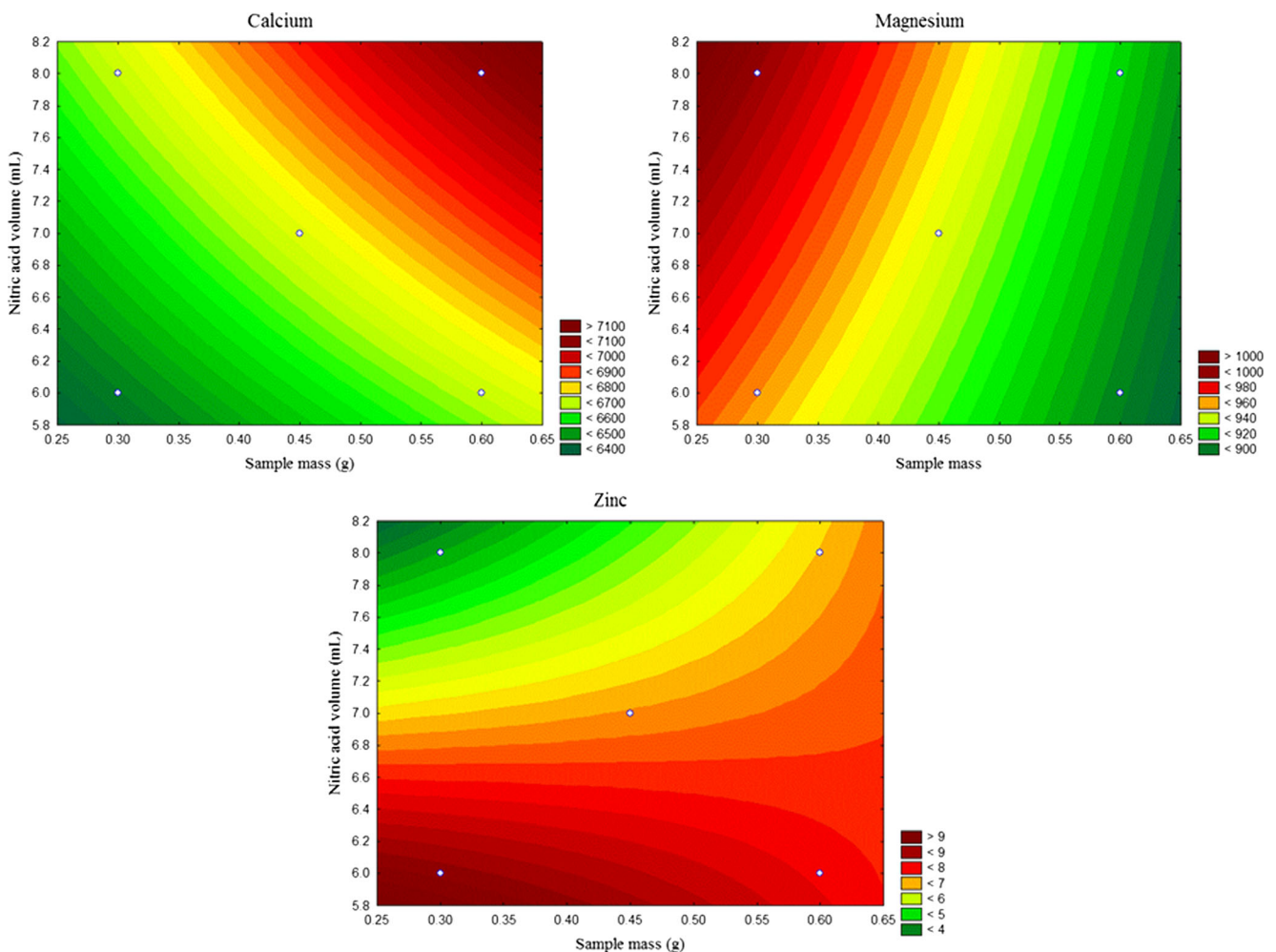
For iron determination, it was observed that no linear or interaction effect was statistically significant ( $p$  value  $>0.05$ ); thus, the variation of values for sample mass and nitric acid volume in the range studied in this work has no effect on the iron concentration, and hence, any one of the assay conditions can be used, prioritizing the one which best fits the data for recovery and repeatability.

According to the Pareto chart, for zinc determination in citrus residue, only the nitric acid volume was significant, indicating that the increase in nitric acid volume decreases zinc concentration, and thus, the adequate way would be to use the lowest values of acid volume, i.e., 6 mL. Sample mass and its interaction with nitric acid volume have no significant influence on zinc concentration. The analysis of variance for zinc concentration indicated that only acid volume was

significant ( $p$  value  $<0.05$ ) and that there was no lack of fit for the model ( $R^2 = 0.70$ ).

For magnesium determination, the variable sample mass presented significant effect on its mineralization ( $p < 0.05$ ), decreasing by  $68.9 \mu\text{g g}^{-1}$  the concentration of magnesium when the sample mass is increased. Nitric acid volume and its interaction with sample mass were not statistically significant ( $p > 0.05$ ). The ANOVA for magnesium mineralization showed no lack of fit, the data being fit to the model. Explained variation ( $R^2$ ) indicates that 87% of the values are explained by the model. Sample mass was significant, and although nitric acid volume is not significant, there is an increasing trend in magnesium concentration with the rise of nitric acid volume during mineralization, as can be observed in the contour curve for magnesium in Fig. 2.

Therefore, according to the assessment of the contour curves and its effects, it was observed that, for iron determination, different volumes and sample masses do not interfere with mineral extraction; for zinc, a decrease in nitric acid volume causes a slight increase in the mineral concentration.



**Fig. 2** Contour curves for calcium, zinc, and magnesium

However, it was observed that in these same conditions, the repeatability for the mineral is not satisfactory; for magnesium analyses, the increase in sample mass decreases its concentration; for calcium, the increase in both sample mass and nitric acid volume improves mineral extraction. Additionally, residual carbon was analyzed for all samples of the experimental design; all presented values below 1%, indicating that there was adequate degradation of organic matter. Visually, all tubes were translucent, with absence of precipitates.

The optimum condition to be selected is the one which not only provides greater recovery of all minerals but also guarantees good repeatability for the method used. Then, the condition considered as the most appropriate for sample preparation of minerals in citrus residue, and which meets these requirements, was that of assay 4, which used the greatest amount of sample (0.6 g) and the highest volume of HNO<sub>3</sub> (8 mL).

### Validation of the Method for Determination of Iron, Calcium, Magnesium, and Zinc by FAAS

For the parameters of linearity and sensitivity, analytical curves were constructed (six to seven points for each element) and linear ranges presented. The concentration values for construction of the analytical curve of each element were as follows: Ca ranged from 0.5 to 5.0 mg L<sup>-1</sup>, Fe from 0.5 to 3 mg L<sup>-1</sup>, Mg from 0.025 to 0.5 mg L<sup>-1</sup>, and Zn from 0.05 to 0.35 mg L<sup>-1</sup>.

The ANOVA of regression for each element showed that the regressions are significant. It is also observed that there is no lack of fit for the models, which indicates both its linearity and the adequacy of the percentage of explained variance ( $R^2 > 0.99$ ) to represent the data. In the residue, the absence of discrepant values was evaluated using Jackknife test and the homogeneity of variance of the residue (homoscedasticity) using Levene's test. It was observed that the values were distributed randomly on the  $x$ -axis, being free of trends.

Precision was evaluated in parameters of repeatability and intermediate precision. For calcium, the mean concentration obtained was  $6.7 \pm 0.2$  mg g<sup>-1</sup>; for iron,  $38.0 \pm 1.8$  µg g<sup>-1</sup>; for magnesium,  $904.7 \pm 35.2$  µg g<sup>-1</sup>; and for zinc,  $7.6 \pm 0.3$  µg g<sup>-1</sup>. According to the AOAC (2002), the values of the coefficient of variation suitable for repeatability must be from half to twice the value of  $C^{-0.15}$  (where  $C$  is the mineral concentration). Then, the coefficients of variation for calcium, iron, magnesium, and zinc should be 1.1–4.2, 2.3–9.2, 1.4–5.7, 2.9–11.7%, respectively. Therefore, the coefficients 3.0, 4.6, 3.9, and 3.5% found for the minerals Ca, Fe, Mg, and Zn, respectively, are within the acceptable range of CV values calculated by AOAC guideline; thus, repeatability was considered fit.

The analysis of variance for intermediate precision was performed on three different days, by the same analyst. The

$p$  value indicates whether the analysis of variance was significant; as  $p$  values for all minerals were higher than 0.05 and calculated  $F$  ( $F_{\text{cal}}$ ) was lower than tabulated  $F$  ( $F_{\text{tab}}$ ) for calcium ( $F_{\text{cal}} = 1.05 < F_{\text{tab}} = 3.55$ ), iron ( $F_{\text{cal}} = 1.69 < F_{\text{tab}} = 3.68$ ), magnesium ( $F_{\text{cal}} = 0.94 < F_{\text{tab}} = 3.68$ ), and zinc ( $F_{\text{cal}} = 3.22 < F_{\text{tab}} = 3.55$ ), it is considered that there was no significant variation of mineral concentrations in citrus residue over the days of analysis; therefore, intermediate precision was also considered fit.

For recovery tests for iron and zinc, the sample was fortified by 50 and 100% regarding the initial value present in the sample; so, the 20 and 40 mg L<sup>-1</sup> sample for iron and the 3.75 and 7.50 mg L<sup>-1</sup> sample for zinc were added before the mineralization. For magnesium and calcium, the sample was fortified with a known concentration (1666.6 mg L<sup>-1</sup>), so as to not interfere with the mineralization for excess of acid volume. Three replicates were made for each mineral concentration.

In Table 4, the percentages of recovery, limits of detection, and quantification for the determination of iron, zinc, calcium, and magnesium in residue of citrus were reported. For the limit of quantification, the values are also presented in micrograms per gram, representing the LOQ and considering sample mass and mineralization dilution.

As suggested by AOAC guidelines (2002), the percentage recovery of iron and magnesium must be between 85 and 110%, 80 and 115% for zinc, and 92 and 105% for calcium. Thereby, recovering the values obtained in the tests, they are within the suggested ranges.

As for LOD and LOQ, considering that in the proposed analytical method a sample mass of 0.6 g is used, with dilution 25 times of the obtained extract, and that the samples have concentrations of approximately 30 µg g<sup>-1</sup> for iron, 7 µg g<sup>-1</sup> for zinc, 6.0 mg g<sup>-1</sup> for calcium, and 800 µg g<sup>-1</sup> for magnesium, the method showed adequate LOD and LOQ to determine these minerals in citrus residue.

### Determination of Iron, Calcium, Magnesium, and Zinc in Citrus Residue

In this step, the content of iron, calcium, magnesium, and zinc was assessed in four different types of citrus residue (Pera orange, Hamlin orange, Sicilian lemon, Tahiti lime) and two types of mixtures (consisting of Pera orange, Hamlin orange, and Tahiti lime). Mineral content was compared among the different types of citrus and in the harvest period, from 3 to 6 months. The data is presented in Table 5.

Based on the mean values presented in Table 5, the element with the highest concentration in citrus residue was calcium, with 6.8 mg g<sup>-1</sup>, the lowest concentration of calcium was observed in Sicilian lemon with 5.5 mg g<sup>-1</sup>, and the highest concentration was observed in mixture 2 with 7.3 mg g<sup>-1</sup>. Only Sicilian lemon was statistically different from the others. The mean iron content level found in the samples was

**Table 4** Recovery, limits of detection, and quantification

Variable	Element			
	Calcium	Iron	Magnesium	Zinc
Recovery (%)	95.4	91.5–105.9	99.8	92.9–99.4
Limit of detection (mg L <sup>-1</sup> )	0.20	0.03	0.01	0.02
Limit of quantification (μg g <sup>-1</sup> )	0,011	0.002	0.005	0.002

116.4 μg g<sup>-1</sup>, ranging from 38.7 to 322.3 μg g<sup>-1</sup>; the highest content level for iron was found in mixture 2 and the lowest in mixture 1. For magnesium, the mean content level was 915.5 μg g<sup>-1</sup>, being the mineral with the second highest concentration, the level ranged from 818.1 μg g<sup>-1</sup> (Sicilian lemon) to 1 mg g<sup>-1</sup> (mixture 2), and the samples of Hamlin orange and mixture 1 were statistically equal to all varieties. Zinc was the mineral found in smaller quantities. The mean zinc concentration was 7.4 μg g<sup>-1</sup>, ranging from 6.5 to 8.2 μg g<sup>-1</sup>; thus, we observed that all samples have statistically equal amounts of this mineral. When comparing all varieties, we

observed that the iron content levels in Pera orange and mixture 1 are statistically equal to Hamlin orange, Tahiti lime, and Sicilian lemon, differing only from mixture 2.

The National Health Surveillance Agency establishes that the Recommended Daily Intake (RDI) for adults is 1000 mg of calcium, 14 mg of iron, 7 mg of zinc, and 260 mg of magnesium (BRASIL 2005); considering the mean content levels obtained from these minerals in citrus, a portion of 100 g of citrus residue would provide 68.3% of RDI for calcium, 35.2% of RDI for magnesium, 83.1% of RDI for iron, and 10.6% of RDI for zinc. According to Resolution no. 27

**Table 5** Content levels of iron, calcium, magnesium, and zinc in citrus in different types of citrus and months of the year

Type of citrus	Element				
		Month	Calcium	Iron	Magnesium
Hamlin orange	May	6.2 ± 0.1b	54.1 ± 5.0a	868.3 ± 18.0b	8.5 ± 0.4a
	June	6.8 ± 0.6b	43.1 ± 7.3a	916.3 ± 7.3a	8.6 ± 1.6a
	July	8.2 ± 0.2a	51.5 ± 3.5a	931.0 ± 17.0a	6.9 ± 0.1a
	Mean	7.0 ± 0.9A	47.9 ± 7.6C	911.7 ± 30.9ABC	8.2 ± 1.3A
Pera orange	August	8.0 ± 0.2a	146.5 ± 5.5a	1110.0 ± 30.8a	8.4 ± 2.0a
	September	6.3 ± 0.1b	87.4 ± 20.7b	945.1 ± 21.6b	6.6 ± 0.1a
	October	6.3 ± 0.4b	58.74 ± 9.9c	937.7 ± 39.5b	7.4 ± 0.3a
	Mean	6.8 ± 0.8A	87.9 ± 39.1BC	982.7 ± 82.9A	7.5 ± 1.1A
Mixture 1		6.6 ± 0.1bAB	38.7 ± 1.6bBC	912.8 ± 10.7bABC	7.7 ± 0.4aA
Mixture 2		7.8 ± 0.2aA	322.3 ± 5.4aA	1006.1 ± 17.2aA	6.5 ± 0.2bA
Tahiti lime	May	5.3 ± 0.1c	96.1 ± 1.6a	794.6 ± 11.4bc	7.8 ± 0.4a
	June	6.2 ± 0.0b	82.5 ± 2.7a	684.1 ± 10.9c	6.9 ± 0.3b
	July	7.7 ± 0.1a	105.9 ± 7.1a	854.3 ± 4.0ab	6.7 ± 0.1bc
	August	8.1 ± 0.3a	231.4 ± 157.2a	931.9 ± 83.4a	6.9 ± 0.4b
	September	7.9 ± 0.1a	109.2 ± 8.6a	928.1 ± 9.8a	8.2 ± 0.3a
	October	8.2 ± 0.1a	131.9 ± 26.8a	906.7 ± 23.9ab	5.9 ± 0.1c
Mean	7.3 ± 1.1A	141.2 ± 99.4B	861.7 ± 98.4BC	7.0 ± 0.8A	
Sicilian lemon	May	5.9 ± 0.2a	75.8 ± 6.1a	832.8 ± 23.9ab	8.7 ± 0.3a
	June	5.8 ± 0.1a	49.4 ± 0.3b	765.0 ± 7.3c	6.3 ± 0.3b
	July	5.3 ± 0.1b	67.9 ± 2.2a	863.3 ± 10.7a	8.1 ± 0.2a
	August	5.1 ± 0.1b	47.7 ± 0.8b	811.3 ± 7.2b	6.3 ± 0.2b
	Mean	5.5 ± 0.4B	60.2 ± 12.9C	818.1 ± 39.3C	7.3 ± 1.1A

Values expressed as mean ± standard deviation ( $n$  = number of samples of each month). Means followed by the same small letters in the column do not differ by Tukey test at the 95% confidence for the same type of citrus. Means followed by the same capital letters in the column do not differ by Tukey test at the 95% confidence for the different type of citrus. For the harvest months,  $n$  = 3 for all samples, except for the month of June of Hamlin orange, October of Pera orange, and August of Tahiti lemon where  $n$  corresponded to six samples. Ca concentration is expressed in milligrams per gram and Fe, Mg, and Zn expressed in micrograms per gram

(BRASIL 1998), a food can be considered source of a mineral when it has at least 15% of the amount recommended by RDI per 100 g of sample. Therefore, citrus residue can be considered a source of calcium, magnesium, and iron. This correlation is important because it shows the mineral nutritional potential of citrus residue, which can be incorporated into other foods or sold separately with greater added value for the producer without burdening the buyer.

Concerning the variation of minerals over the months, it was observed that for Hamlin orange the minerals iron and zinc showed no significant differences over the months. For calcium, only in the month of July was the mineral content different, whereas for magnesium, only in the month of May.

For Pera orange, the variation over the months was more pronounced, showing significant differences for iron content, which ranged from 58.7 to 146.5  $\mu\text{g g}^{-1}$ . The content levels for calcium and magnesium in the month of September were statistically equal to those of August. Zinc content level was the same in all months.

When comparing the mixtures, it can be said that they are statistically different in their mineral composition, with mixture 2 having the highest iron, calcium, and magnesium content levels; only the mean zinc content level is lower compared to mixture 1.

Tahiti lime presented no significant variations for iron content over the months. Samples from July to October have calcium values equal to the 95% confidence. Magnesium concentration ranged from 684.1 to 931.9  $\mu\text{g g}^{-1}$ , with July having the lowest percentage for this mineral.

The analysis of Sicilian lemon showed variation from 47.7 to 75.8  $\mu\text{g g}^{-1}$  for iron and 6.3 to 8.7  $\mu\text{g g}^{-1}$  for zinc. Thus, the months of May and July were both equal to each other and different from the others. In relation to calcium content, the months of May and June are equal and present the highest means for the mineral. For magnesium, the months of June, July, and August were different.

Barros et al. (2012) determined some minerals in peels of Pera orange and Tahiti lime (with 66.6 and 72.6% of moisture, respectively). Regarding the results found in this study, the content levels of calcium, iron, and magnesium in orange residue were higher than those found in peels by Barros et al. (2012), except for zinc content, which was lower. For Tahiti lime, the values of peels and residues for calcium and zinc concentration are close, the iron content levels of this study are higher, and the magnesium content levels are lower than those in Barros et al. (2012).

Xu et al. (2008) evaluated peels of another type of citrus and found values of Ca and Mg close to this study, while the contents of Fe were lower (3.78–26.74  $\mu\text{g g}^{-1}$ ) and Zn contents were higher than those of citrus residues for one of the varieties studied by them (28.15  $\mu\text{g g}^{-1}$ ). Gondim et al. (2005) found tangerine contents of Ca and Fe to be similar to those of

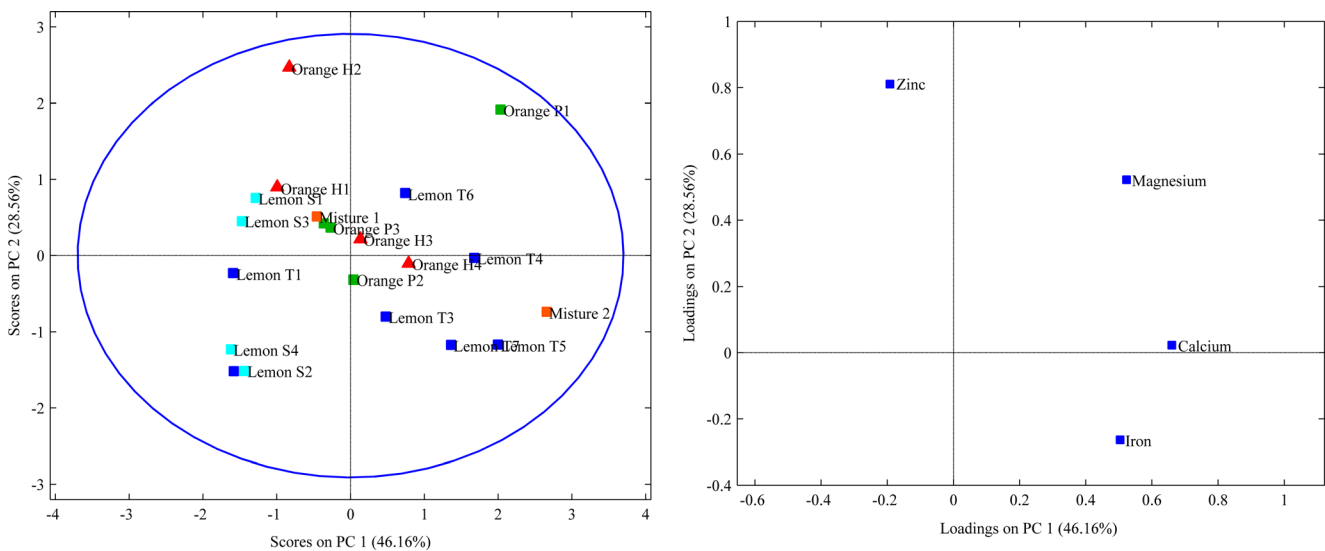
citrus residue of this study as well as higher contents of Mg and Zn. Dhiman et al. (2011) evaluated oranges of India and observed mean contents of Zn and Mg in peels higher than and contents of Ca lower than those of citrus residue of this study.

In order to provide a better visualization of the obtained data, the principal component analysis (PCA) was carried out. For the construction of PCA, we used 21 samples of citrus residue produced in different months and 4 types of minerals Ca, Fe, Mg, Zn, resulting in a  $21 \times 4$  matrix. The data matrix ( $21 \times 4$ ) was constructed using samples as lines and minerals as columns. The data was auto-scaled, since we intended to assign the same value to all variables, which had different magnitudes. Figure 3 presents the graphs for scores, loadings, and explained variance by main components. The first and second main components were chosen because they represent a total explained variance of 74.72%.

For PC1, the variables of greatest importance were iron, calcium, and magnesium, while for PC2, the variable with greatest explained variation was zinc, followed by magnesium. The score graph shows the distribution of the samples and correlates them to the loading graph, which shows the influence of each mineral on the separation of the citrus samples. We observed that PC2 separates the samples mainly according to the content of the mineral zinc; hence, samples rich in calcium, iron, and magnesium were closer and samples with smaller quantities of these minerals were more distant. The sample Orange H2, for example, has the highest zinc content level and low iron concentration; the sample M2 has high content levels of iron, calcium, and magnesium. The group of samples identified as lemon T1 and T2 as well as lemon S2 and S4 have the lowest mean concentrations of calcium, iron, magnesium, and zinc.

Exploratory principal component analysis did not separate the samples in groups (orange, lemon, or mixture), which means that the samples may vary their mineral content among the types of citrus and over different times of the year. However, this variation is not significant to separate the samples into distinct groups. Then, it can be said that there is certain homogeneity of the samples as for their mineral content. Thus, orange or lemon residue has the potential to be included in food product formulations or to be sold separately, maintaining similar mean values for Ca, Fe, Zn, and Mg over the year and among the different types of citrus, which implies the possibility of citrus residue exploration from residue of orange and lemon juice industries, its use in human food rather than being largely directed to animal feed industry, and the possibility of its being a product rich primarily in Ca and Fe, contributing to the decrease in the deficiency of these minerals in human food.





**Fig. 3** Graphs of scores (a) and loadings (b) for the citrus samples. Hamlin orange samples collected in the months of May, June, and July are represented by Orange *H1*, *H2*, and *H3*. Pera orange waste samples collected in the months of August, September, and October are

represented by Orange *P1*, *P2*, and *P3*. Tahiti lime waste samples from May to October are represented by lemon *T1*, *T2*, *T3*, *T4*, *T5*, and *T6*, and Sicilian lemon from May to August by lemon *S1*, *S2*, *S3*, and *S4*. Mixture 1 is *M1* and mixture 2 is *M2*

## Conclusion

The experimental design proved to be adequate to optimize the conditions for sample mineralization to obtain the highest concentrations of each mineral and repeatability. The method presented fit linearity, selectivity, precision, limit of detection, and limit of quantification for analysis of iron, calcium, magnesium, and zinc in dry samples of residue from different types of citrus. In addition, it presents advantages such as low cost, simplicity, and the possibility of obtaining a suitable condition to evaluate the studied minerals in a reliable way.

It is concluded that citrus residue can be considered a good source of minerals Ca, Fe, and Mg, as well as Zn, with excellent contribution to Fe and Ca RDI especially, and even with certain variation of mineral concentration over the harvest period, they remained as sources of these minerals. Besides, groupings among the different matrices were not observed. Thus, the use of dry citrus residue as ingredient in food formulations becomes viable to add nutritional value to the products.

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## Compliance with Ethical Standards

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**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed Consent** Not applicable.

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