



## Multivariate optimization of an ultrasound-assisted extraction procedure for Cu, Mn, Ni and Zn determination in ration to chickens

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### ABSTRACT

In this work, multivariate optimization techniques were used to develop a method based on the ultrasound-assisted extraction for copper, manganese, nickel and zinc determination from rations for chicken nutrition using flame atomic absorption spectrometry. The proportions of extracting components (2.0 mol.L<sup>-1</sup> nitric, hydrochloric and acetic acid solutions) were optimized using centroid-simplex mixture design. The optimum proportions of this mixture taken as percentage of each component were respectively 20%, 37% and 43%. Variables of method (sample mass, sonication time and final acid concentration) were optimized using Doehlert design. The optimum values found for these variables were respectively 0.24 g, 18s and 3.6 mol.L<sup>-1</sup>. The developed method allows copper, manganese, nickel and zinc determination with quantification limits of 2.82; 4.52; 10.7; e 9.69 µg.g<sup>-1</sup>, and precision expressed as relative standard deviation (%RSD, 25 µg.g<sup>-1</sup>, N = 5) of 5.30; 2.13; 0.88; and 0.83%, respectively. This method was applied in the analytes determination from chicken rations collected from specialized commerce in Jequié city (Bahia State/Brazil). Application of paired t-test at the obtained results, in a confidence level of 95%, does not show significant difference between the proposed method and the microwave-assisted digestion.

**Key words:** rations to chicken, ultrasound-assisted extraction, mixture design, Doehlert design, metals.

### INTRODUCTION

Nowadays, Brazil is a major producer and exporter of chicken meat, and in 2007 produced 10.3 million tons and exported 3.2 million for foreign market. In the last decade, the domestic consumption of chicken meat in the country exceeded the consumption of other meats, such as beef and pork.

Therefore, assessing factors such as adequate food for birds is very important to improve the quality of the produced meat (Roppa 2008).

The use of rations for nutrition of chickens destined for meats and eggs production is an economical, efficient and rational way of providing all the nutrients in a balanced way to these animals. Macro (Ca, Mg, P, K, Na, Cl and S) and micro nutrients (Cu, Co, Zn, Fe, Ni, Mn, I, Mo, Se and

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Cr) must be present in rations in adequate amounts, avoiding risks of malnutrition, which would be difficult to control using other types of food. Determining mineral content in rations for animal nutrition is of great importance in assessing its quality, mainly due to the necessity of verifying the quantities of mineral nutrients supplied by these rations and evaluating the possibility of contamination by toxic metals (such as Pb, Hg and Cd) that can often be present in rations (Edwards and Veltman 1983).

The search for new procedures that are quick, easy to perform and that require smaller quantities of reagents has been a major driver of research in the field of Analytical Chemistry in the recent years. Within this context, the energy present in the ultrasonic waves has been used for developing methodologies for extraction of chemical species in different types of samples (Mason and Lorimer 2002).

Ultrasounds are mechanical waves that propagate in material media, with a speed that can vary from hundreds of meters per second in the air up to thousands of meters per second in solids. That occurs in consecutive cycles of compression and rarefaction at frequencies higher than the audible frequencies for humans, which is above 16 KHz (Korn et al. 2003).

Ultrasound-assisted leaching is considered by many authors an efficient way to extract metal species present in different types of samples and constitute an environmentally friendly technique (Luque-Garcia and Castro 2003, Magalhães et al. 2009). One can mention the following works that use the ultrasound energy for extracting metals: determining of Ca, Mg, Mn and Zn in plant tissue samples (Nascentes et al. 2001); Ca, Mg, Mn and Zn in samples of fish ration (Neves et al. 2009); electrolytic manganese in slag (Li et al. 2008); Mg, Mn and Zn in plant tissue samples (Filgueiras et al. 2000); cadmium and lead in mussel samples (Biurrun et al. 2005). In these papers FAAS was used as detection technique. One can also mention the Hg determination in soil,

river and sea sediments (Collasiol et al. 2004); Cd and Pb present in plant leaves (Ruiz-Jimenez et al. 2003) using graphite furnace atomizer in AAS; trace elements in hair samples by inductively coupled plasma mass spectrometry (ICP-MS) (Batista et al. 2009); determination of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in sewage sludge (Hristozov et al. 2004) and metals in edible seaweed (Gonzalez et al. 2005) using inductively coupled plasma optical emission spectrometry (ICP OES).

In this paper, it was applied multivariate optimization techniques (mixture and Doehlert designs) to develop an analytical method, using ultrasound-assisted extraction of Cu, Mn, Ni and Zn in rations used for chicken nutrition and determination using flame atomic absorption spectroscopy (FAAS).

## MATERIALS AND METHODS

### INSTRUMENTATION

A Perkin Elmer (Norwalk, CT, USA) Analyst model 200 flame atomic absorption spectrometer equipped with a deuterium lamp for background correction was used for measuring absorbance. Hollow cathode lamps for copper, manganese, nickel and zinc were used in accordance with the recommendations of the manufacturer (0.7, 0.2, 0.2, and 0.7, for bandwidth of the slit, 324.8, 279.5, 232.0, and 213.9 nm for wavelengths to Cu, Mn, Ni and Zn, respectively). The burner height (13.5 mm) was also used with conventional values. Flame was consisted of acetylene (flow rate 2.0 L.min<sup>-1</sup>) and air (flow rate 13.5 L.min<sup>-1</sup>). Flow rate used for the nebulizer was 5.0 mL.min<sup>-1</sup>.

A Unique (São Paulo, Brazil) ultrasonic bath, Maxiclean 1450 model was used to promote the extraction of target elements. The ultrasound bath equipment was filled with 2.8 L of water and one-by-time glass centrifuge tube was placed, with the aid of a base, in the center of the bath to perform the ultrasound assisted extraction.

A QUIMIS T 222 QM centrifuge (São Paulo, Brazil) with 50 mL centrifuge tubes was used to separate solid from liquid-phase. A Sartorius (model BL D105) analytical balance was used to establish the sample mass. Digestion of ration samples was carried out in a Parr 4749 (Moline, IL, USA) digestion bomb enclosing a chemically inert Teflon sample cup of 23 mL.

#### REAGENTS AND SOLUTIONS

All chemicals and reagents used in this study were of analytical-reagent grade. Ultrapure water (Milli-Q<sup>®</sup>, Millipore, USA) with resistivity of 18.2 mΩ cm was used throughout. The laboratory glassware was kept in diluted nitric acid at least overnight, and subsequently washed with deionized water. Solutions of 10% (m/v) Triton X-100 surfactant (Sigma-Aldrich, Milwaukee, USA) were prepared in high purity deionized water. One thousand milligrams per liter stock standard solutions of Cu, Mn, Ni and Zn were purchased from Merck (Darmstadt, Germany). Nitric and hydrochloric acid solutions were prepared by direct dilution with deionized water from the concentrated solutions (Merck, Darmstadt, Germany).

#### COLLECTION OF SAMPLES

Samples of chicken ration used were collected in the trade of Jequié city, Bahia, Brazil. Samples were stored on their own sale packaging and transported to the laboratory, where they were subjected to preliminary treatments. Rations were collected for three different purposes (promote growth, fattening, and laying eggs).

#### PRELIMINARY TREATMENTS OF SAMPLES

Samples were taken to oven and dried at a temperature of 70°C until all moisture was removed. After drying, samples were left in order to reach room temperature to be crushed in a knives mill. Comminuted samples were sieved through 300

µm sieves and later 100 µm and then stored in polyethylene bottles within dissectors. The remaining fractions were comminuted and sieved again, until those samples do not present some segregation.

#### TOTAL DIGESTION OF CHICKEN RATION SAMPLES IN MICROWAVE OVEN

Ration samples (0.1 g) were digested by adding 2.0 mL of 1:1 (v/v) nitric acid solution to the sample. A Teflon cup was placed in a PARR 4781 digestion bomb and heated in a microwave system for 1 min at 264.9 W. Blank samples were prepared in the same way. Subsequently, samples were cooled to room temperature, transferred to a volumetric flask and diluted to a final volume of 10 mL with deionized water. Metals were determined in the final solution by flame atomic absorption spectrometry (FAAS). Assays of metals found in the samples using the microwave radiation-assisted acid digestion method were taken as reference values (Santos et al. 2009) in establishing the percentage of extracted metals in relation to the method of ultrasound-assisted extraction.

#### OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION PROCEDURE

To optimize the ultrasound-assisted extraction method of metals (Cu, Mn, Ni and Zn) in ration samples for chicken nutrition, we used two types of experimental design: a simplex centroid mixture design (Bruns et al. 2006, Massart et al. 1997) to optimize the ratio of extracting solutions (HCl, HNO<sub>3</sub> and H<sub>3</sub>COOH), and a Doehlert design (Ferreira et al. 2007) for optimization of method variables (final concentration of acid, sample mass, and sonication time).

To allow the simultaneous optimization of the four obtained responses (percentage of metal extraction), a mathematical approach developed by Derringer and Suich was used (Derringer and Suich 1980). It is based on desirability functions applied in optimizing multi-response experiments.

The approach consists of first converting each response  $y_i$  into an individual desirability function  $d_i$ , which varies within the range  $0 \leq d_i \leq 1$ . If the response is the desired,  $d_i = 1$ , and if the response is outside the acceptable region,  $d = 0$ . Thus, the factor levels are chosen to maximize the overall desirability given by the expression:

$$D = \sqrt[m]{d_1 d_2 \dots d_m} \quad (\text{Eq. 1})$$

Where  $m$  is the number of response variables. For example: if the target value  $T$  is a **maximum**, there will be:

$$d = \begin{cases} 0 & \text{if } y < L \\ \left(\frac{y-L}{T-L}\right)^s & \text{if } L \leq y \leq T \\ 1 & \text{if } y > T \end{cases} \quad (\text{Eq. 2})$$

where  $L$  is the lowest acceptable value for the response, and  $s$  is the weight (when equal to 1 = linear desirability function). In this work,  $L$  values were the lowest recovery found in a set of experiments for each metal and  $T$  values were the highest recovery also for each metal.

#### *Use of Mixture Design in the Optimization of Extracting Acid Solutions Proportions*

The following procedure was followed: about 0.3 g of chicken ration sample was weighed directly into a 50 mL dry centrifuge tube. Later, different volumes of each extracting solution were added in order to always have a final volume of 6.0 mL. The proportion of each acid solution in each experiment is established by the mixture design matrix used (centroid simplex design). In this step, variables of the method such as acid concentration, sample mass and sonication time were fixed at 2.0 mol.L<sup>-1</sup>, 0.3 g and 15 minutes, respectively.

#### *Use of Doehlert Design in the Optimization of Method Variables*

Variables of the method to be optimized in this stage were: acid concentration (AC) in mol.L<sup>-1</sup>, sample mass (SM) in grams, and sonication time

(ST) in minutes. Due to the characteristics of the experimental design matrix used in this study, AC and SM were studied in five levels and ST in three levels. The acid proportions considered as optimal in the previous mixture design were used.

The following procedure was followed: 0.1 to 0.5 g of chicken ration sample was weighed directly into a 50 mL dry centrifuge tube. Later, volumes considered as optimal from each of the extracting solutions were added. The concentration of acids, sample mass, and sonication time corresponding to each experiment are set by the design matrix.

## RESULTS AND DISCUSSION

The development of fast and accurate methods to allow determining amounts of micro nutrients such as Cu, Mn, Ni and Zn in rations for animal nutrition is of concern due the necessity of monitoring their levels and to ensure that metals are offered in adequate concentration to the chickens. Low concentrations of these metals cause malnutrition but, on the other hand, high levels can be toxic and affect the bird health decreasing the performance of the creation. Thus, quality control protocols based on reliable analytical methods must be implemented to guarantee the food security of population.

### SIMPLEX CENTROID MIXTURE DESIGN

Simplex centroid design is very useful in establishing optimal proportions among the various components of a mixture. In ultrasound-assisted extraction, acid solutions are usually used as extracting agents due to their good efficiency. This efficiency can be improved by using mixtures of these solutions. However, it is necessary to use an appropriate proportion of these extractors to achieve a good extraction and improve the analytical characteristics of the method.

The experimental simplex centroid design used, the recoveries for the studied metals, and the overall desirability are shown in Table I.

All recoveries, and consequently all the overall desirability, were obtained in duplicate for each experiment in order to estimate the experimental error.

Linear, quadratic, special cubic, and full cubic mathematical models were fitted to the data generated for overall desirability to obtain the

**TABLE I**  
**Mixtures design matrix and responses (recovery percentage of metals) to optimize the proportions of extracting solutions of nitric acid (HNO<sub>3</sub>), hydrochloric acid (HCl) and acetic acid (HAc).**

Variables			Extraction (%)				Overall Desirability
HNO <sub>3</sub>	HCl	HAc	Cu	Mn	Ni	Zn	
6 (1)	0 (0)	0 (0)	81.01 / 86.00	83.21 / 88.77	46.23 / 51.85	99.54 / 97.11	0.3809 / 0.4962
0 (0)	6 (1)	0 (0)	74.34 / 60.07	81.51 / 71.38	78.34 / 78.34	85.04 / 81.98	0.2820 / 0.0000
0 (0)	0 (0)	6 (1)	62.63 / 62.98	90.33 / 88.35	39.50 / 45.16	87.74 / 88.22	0.0000 / 0.1882
3 (1/2)	3 (1/2)	0 (0)	73.44 / 76.62	82.79 / 82.05	45.63 / 65.12	95.14 / 92.01	0.3067 / 0.4251
3 (1/2)	0 (0)	3 (1/2)	97.27 / 89.42	97.91 / 97.68	52.69 / 58.45	101.3 / 97.05	0.6523 / 0.6312
0 (0)	3 (1/2)	3 (1/2)	80.62 / 78.43	90.83 / 94.11	85.44 / 97.91	95.07 / 92.48	0.6449 / 0.6552
2 (1/3)	2 (1/3)	2 (1/3)	86.73 / 84.43	91.64 / 98.84	78.10 / 78.10	100.2 / 97.17	0.6544 / 0.7827
4 (4/6)	1(1/6)	1(1/6)	84.46 / 84.54	92.57 / 96.71	58.39 / 58.45	99.22 / 92.94	0.5900 / 0.5117
1(1/6)	4 (4/6)	1(1/6)	82.32 / 78.43	88.76 / 89.07	105.2 / 97.92	97.35 / 94.34	0.7282 / 0.6410
1(1/6)	1(1/6)	4 (4/6)	85.44 / 89.00	96.59 / 100.76	59.07 / 58.17	97.15 / 98.27	0.6080 / 0.6569

Note: The coded values are given in brackets.

best description of the experimental region. To assess the quality of the model, the lack of fit was verified. If the mathematical model is well fitted to the experimental data, the mean square of lack of fit (MS<sub>lof</sub>) should reflect only the random errors inherent to the system. Additionally, mean square of pure error (MS<sub>pe</sub>) should also be an estimative of these errors and it is assumed that these two values are not statistically different. Thus, it is possible to use the *F* distribution to assess whether there is any statistical difference between these two means.

According to Analysis of Variance (Anova) presented in Table II, the linear model showed lack of fit ( $F_{\text{calculated}} > F_{\text{tabled}}$ ) and left large residues. Therefore, it presents low predictive capability. The quadratic model did not show lack of fit ( $F_{\text{calculated}} < F_{\text{tabled}}$ ), left smaller residues and, therefore, has a good predictive capability. The special cubic and full cubic models also left low residues, but the small improvement in predictive capability is not a rationale for the use of a more complex model.

Thus, it was decided to use the quadratic model to the description of the studied experimental region.

The following quadratic equation illustrates the relation among the three component volumes of the studied mixtures and overall desirability (OD):

$$\begin{aligned}
 OD = & 0.408V_{HNO_3} + 0.173V_{HCl} + \mathbf{0.009} V_{HAc} \\
 & (\pm 0.07) \quad (\pm 0.07) \quad (\pm 0.07) \\
 & + \mathbf{0.459} V_{HNO_3}V_{HCl} + 1.6V_{HNO_3}V_{HCl} \\
 & (\pm 0.3) \quad (\pm 0.3) \\
 & + 2.3V_{HCl}V_{HAc} \quad \quad \quad (Eq. 3) \\
 & (\pm 0.3)
 \end{aligned}$$

In equation 3, terms in bold are not significant. They were not eliminated from the equation to allow calculation of the critical point. In Figure 1a and 1b, response surface and the contour plot described by equation 1 are presented and show the behavior of response with the level variation of the studied factors.

Figure 2 shows the individual desirability profiles (related to each metal) and the overall

**TABLE II**  
**Analysis of variance (ANOVA) for linear, quadratic, special cubic, and full cubic models, fitted to overall desirability, with a 95% confidence level.**

<b>Linear Model</b>					
Source of Variation	SS	df	MS	F <sub>calculated</sub>	F <sub>tabled</sub>
Model	0.0363	2	0.0181	0.318	3.59
Total error	0.970	17	0.0571		
Lack of fit	0.884	7	0.126	14.79	3.14
Pure error	0.085	10	0.008		
<b>Quadratic Model</b>					
Source of Variation	SS	df	MS	F <sub>calculated</sub>	F <sub>tabled</sub>
Model	0.860	5	0.172	16.48	2.96
Total error	0.146	14	0.010		
Lack of fit	0.054	4	0.014	1.59	3.48
Pure error	0.085	10	0.008		
<b>Special Cubic Model</b>					
Source of Variation	SS	df	MS	F <sub>calculated</sub>	F <sub>tabled</sub>
Model	0.870	6	0.145	13.8	2.92
Total error	0.137	13	0.0105		
Lack of fit	0.045	3	0.015	1.72	3.71
Pure error	0.085	10	0.008		
<b>Full Cubic Model</b>					
Source of Variation	SS	df	MS	F <sub>calculated</sub>	F <sub>tabled</sub>
Model	0.913	8	0.114	13.5	2.95
Total error	0.0932	11	0.00848		
Lack of fit	0.007	1	0.007	0.87	4.96
Pure error	0.085	10	0.008		

SS, sum of square; df, degree of freedom; MS, mean of square.

desirability profile indicating the optimum proportions of each component. The fourth column of graphics, presented in Figure 2, shows how the profiles vary from individual desirability for each response according to the limits established for *L*, *T* and *s* in equation 1. Since *L* was the lowest value found for each set of responses, *T* was the highest of them and *s* was equal to 1, the graphics present linear profiles.

Using overall desirability as response, it possible to see that there is a maximum as critical point. The coordinates of these points are the proportions of components that generate the highest

response for the studied system. Thus, analyzing the desirability profile graph, the optimal values found to performing simultaneous extractions of studied metals were: 1.2 mL (20%) of HNO<sub>3</sub>, 2.2 (37%) of HCl, and 2.6 (43%) of HAc.

#### *Doehlert Design*

The performance of ultrasound-assisted extraction depends on proportions of acid solutions used to extract analytes, as well as some method variables such as particle size, acid concentration, sample mass, sonication time, extractor volume, among others.

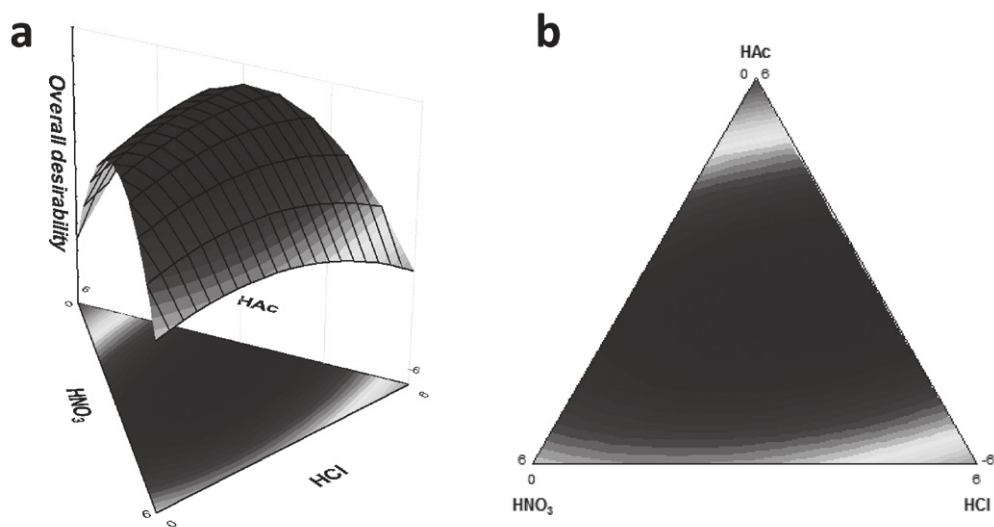


Figure 1 - (a) Response surface and (b) contour graph for optimization of extraction proportions in an ultrasound extraction procedure using centroid mixture design.

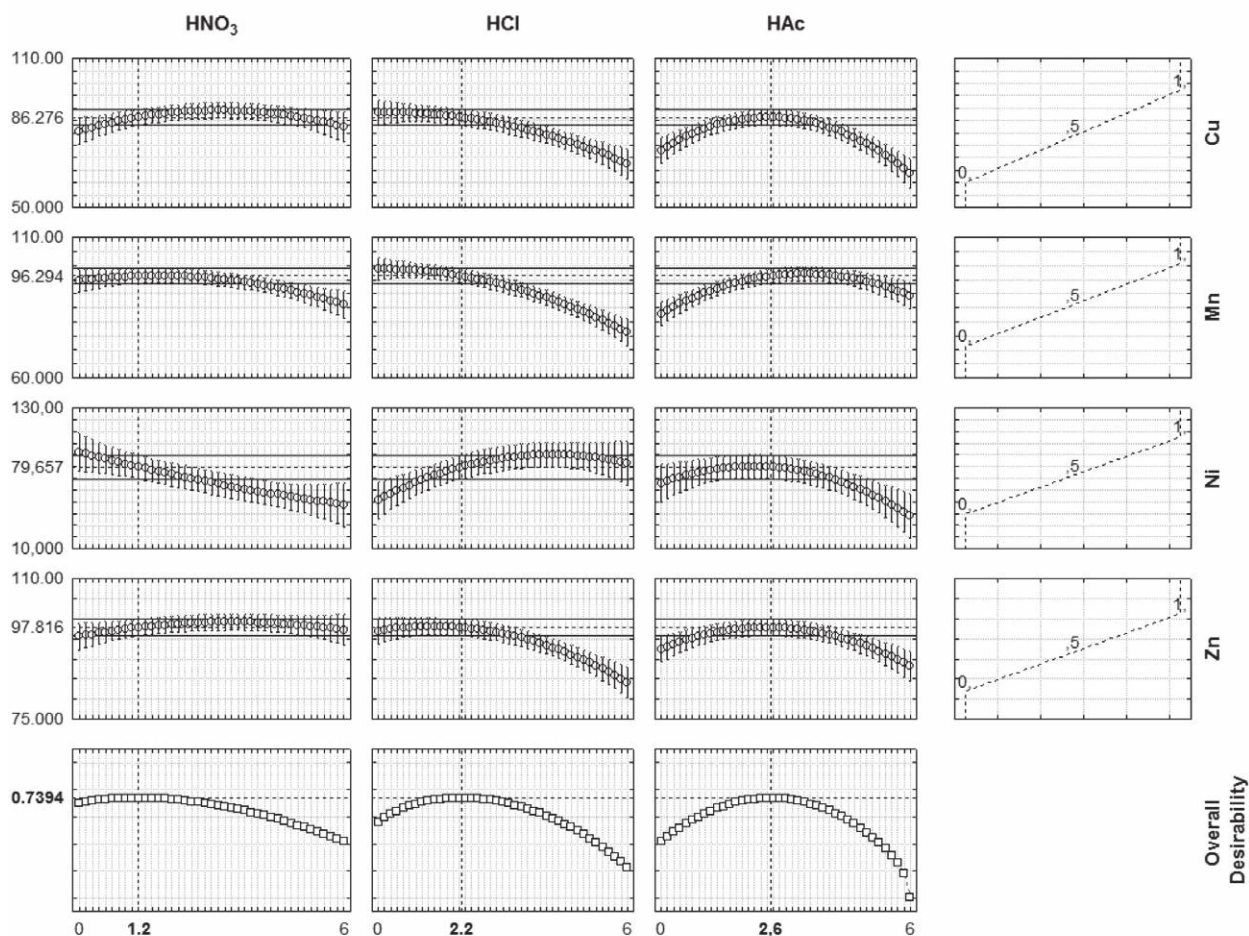


Figure 2 - Profiles for predictive values, individual and overall desirability in optimizing the proportions of the mixture variables.

The experimental Doehlert design used, recoveries for the studied metals, and the overall desirability are shown in Table III. For description of the experimental area, the overall desirability was once again used as response, in order to obtain a simultaneous optimization of the four metals studied. According to the ANOVA Table (Table IV), both linear and quadratic model showed lack of fit ( $F_{\text{calculated}} > F_{\text{tabled}}$ ). In spite of the quadratic model presents lack of fit, it leaves lower residue values than the linear and it was used to indicate the optimum experimental conditions. Experiments were performed using these conditions and it was verified that there were quantitative recoveries for

all studied metals. The quadratic equation shown below was found:

$$\begin{aligned}
 OD = & 0.756 - \mathbf{0.003(AC)} - 0.148(AC)^2 - 0.261(SM) \\
 & (\pm 0.05) \quad (\pm 0.03) \quad (\pm 0.06) \quad (\pm 0.03) \\
 & - 0.518(SM)^2 + 0.142(ST) - 0.481(ST)^2 \\
 & (\pm 0.06) \quad (\pm 0.03) \quad (\pm 0.07) \\
 & - \mathbf{0.132(AC)(SM)} - \mathbf{0.041(AC)(ST)} \\
 & (\pm 0.07) \quad (\pm 0.05) \\
 & - \mathbf{0.016(SM)(ST)} \quad (\text{Eq. 4}) \\
 & (\pm 0.05)
 \end{aligned}$$

Again, in equation 4, terms in bold are not significant, but they were not eliminated from the equation to allow calculation of the critical point.

TABLE III  
Doehlert design and responses (recovery percentage of copper, manganese, nickel, and zinc) for optimization of variables method: acid concentration (AC), sample mass (SM), and sonication time (ST).

Variables			Extraction (%)				Overall Desirability
AC	SM	ST	Cu	Mn	Ni	Zn	
3 (0)	0,5 (1)	15 (0)	71.08 / 64.44	79.60 / 76.98	44.33 / 53.05	61.95 / 62.75	0.0991 / 0.1299
2 (-0.5)	0,4 (0.5)	5 (-0.707)	48.33 / 51.79	77.65 / 76.43	60.78 / 54.67	73.58 / 75.76	0.0000 / 0.1554
2 (-0.5)	0,4 (0.5)	25 (0.707)	66.56 / 62.74	90.63 / 80.87	69.83 / 61.09	75.38 / 78.03	0.4290 / 0.3145
4 (0.5)	0,4 (0.5)	5 (-0.707)	70.49 / 63.70	73.28 / 75.39	44.21 / 43.70	68.10 / 61.79	0.0035 / 0.0612
4 (0.5)	0,4 (0.5)	25 (0.707)	100.8 / 100.75	79.16 / 75.91	64.83 / 66.35	61.28 / 64.41	0.0000 / 0.2318
1 (-1)	0,3 (0)	15 (0)	86.27 / 90.84	91.67 / 91.65	66.58 / 58.65	78.92 / 81.54	0.5369 / 0.5223
3 (0)	0,3 (0)	15 (0)	82.10 / 72.69	98.19 / 98.76	94.47 / 88.65	98.37 / 92.22	0.8124 / 0.6993
5 (1)	0,3 (0)	15 (0)	101.9 / 100.79	82.12 / 83.63	95.63 / 109.18	82.89 / 94.90	0.6179 / 0.7546
2 (-0.5)	0,2 (-0.5)	5 (-0.707)	75.76 / 68.66	91.32 / 86.89	65.65 / 66.50	79.90 / 84.50	0.4949 / 0.4561
2 (-0.5)	0,2 (-0.5)	25 (0.707)	79.55 / 86.79	93.88 / 89.41	83.15 / 62.13	94.91 / 93.79	0.6989 / 0.5799
4 (0.5)	0,2 (-0.5)	5 (-0.707)	76.40 / 69.85	83.32 / 83.86	62.89 / 63.88	76.85 / 83.36	0.3994 / 0.4178
4 (0.5)	0,2 (-0.5)	25 (0.707)	88.20 / 84.61	89.43 / 82.68	88.01 / 99.50	88.54 / 96.68	0.6815 / 0.6552
3 (0)	0,1 (-1)	15 (0)	79.47 / 81.06	101.6 / 91.31	43.62 / 40.44	89.04 / 90.54	0.3765 / 0.3450

Note: The coded values are given in brackets.

Figure 3 shows the individual and the overall desirability profiles. As it was carried out to calculate the individual desirability for mixture,  $L$  was the lowest value found for each set of responses,  $T$  was the highest of these set and  $s$  was equal to 1. Analyzing this graph, indicating that higher responses are obtained with an acid concentration of 3.2 mol.L<sup>-1</sup>, sample mass of 0.24 g, and sonication time of 18 min.

#### ANALYTICAL FEATURES OF THE PROCEDURE

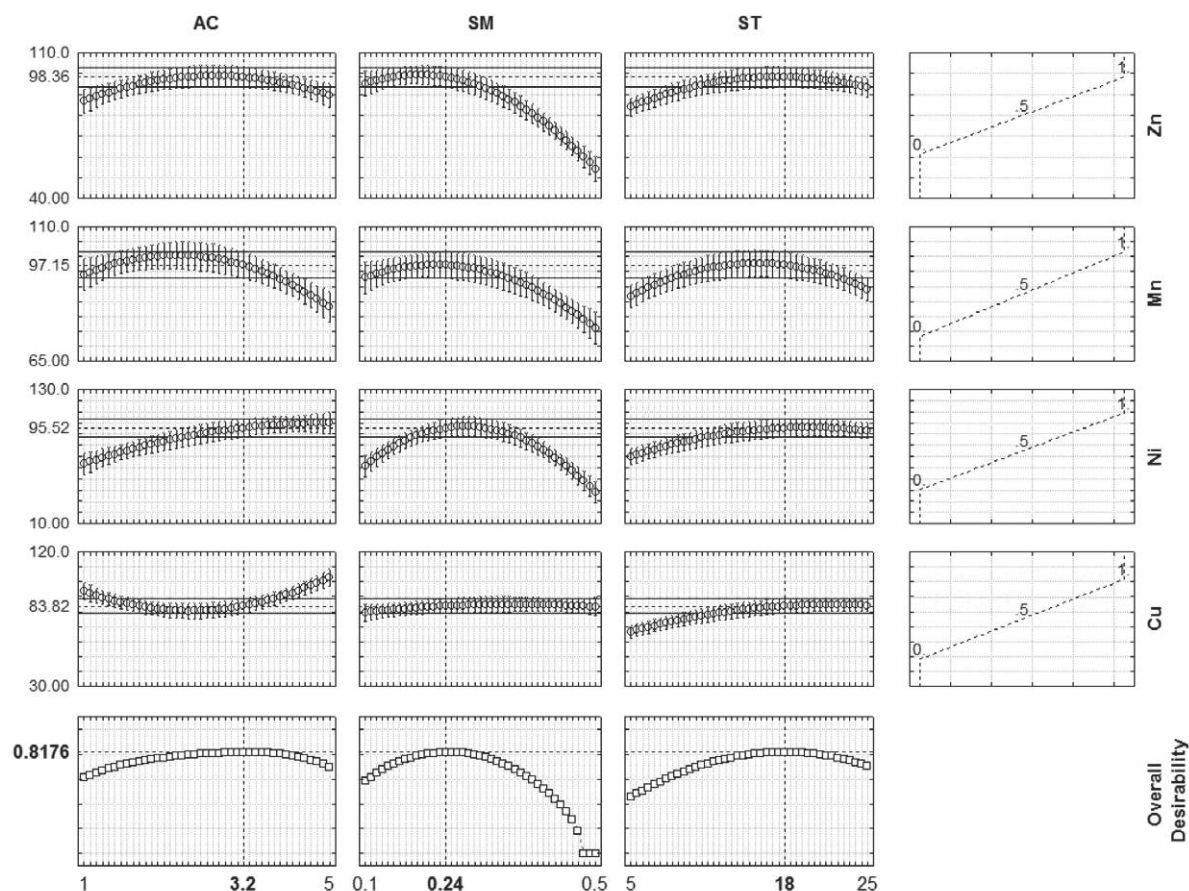
The limit of detection (LOD) was calculated based on three times (3 s) of standard deviation of blank signal by 10 replicate measurements. The limit of quantification (LOQ) is the concentration that gives a response equivalent to 10 times the standard deviation of blank signal ( $n = 10$ ), and it defines the



**TABLE IV**  
**Analysis of variance (ANOVA) for linear and quadratic models**  
**fitted to overall desirability, with a 95% confidence level.**

Linear Model					
Source of Variation	SS	df	MS	F <sub>calculated</sub>	F <sub>tabled</sub>
Regression	0.705	3	0.235	5.77	3.05
Residual	0.895	22	0.0407		
Lack of fit	0.823	9	0.0915	16.4	2.71
Pure error	0.0723	13	0.00556		
Total	1.60	25			
Quadratic Model					
Source of Variation	SS	df	MS	F <sub>calculated</sub>	F <sub>tabled</sub>
Regression	1.30	9	0.144	7.68	2.54
Residual	0.301	16	0.0188		
Lack of fit	0.228	3	0.0761	13.7	3.41
Pure error	0.0723	13	0.00556		
Total	1.60	25			

SS, sum of square; df, degree of freedom; MS, mean of square.



**Figure 3** - Profiles for predictive values, individual and overall desirability in optimizing the values of the method variables.

lower limit of the range. For the proposed procedure, LODs were 0.85, 1.28, 3.50, and 2.90  $\mu\text{g}\cdot\text{g}^{-1}$ , and LOQs were 2.82, 4.52, 10.7, and 9.69  $\mu\text{g}\cdot\text{g}^{-1}$ , and precision expressed as relative standard deviation (%RSD,  $N = 5$ ) of 8.30, 2.13, 0.88, and 0.83% for copper, manganese, nickel and zinc, respectively. Relative standard deviation was determinate with data originated after perform five extractions from a sample that was spiked to a final concentration of 25  $\mu\text{g}\cdot\text{g}^{-1}$ . Additionally, it was verified that Triton X-100 surfactant solutions contribute to increase the blank signal and, consequently, the limit of detection for zinc.

#### APPLICATION AND ACCURACY

After defining the optimal conditions for ultrasound-assisted extraction of the elements studies from rations for chicken nutrition, the developed method was applied to the analysis of nine samples collected in the specialized market of Jequié city, at Bahia State.

The accuracy of the developed procedure was verified by comparing the generated results with those of microwave-assisted digestion for three samples. The results of this analysis are presented in Table V.

**TABLE V**  
Concentrations ( $\mu\text{g}\cdot\text{g}^{-1}$ ) of copper, manganese, nickel and zinc found in samples of rations analyzed using the Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Digestion (MAD).

Kind of ration	Sample	Cu		Mn		Ni		Zn	
		UAE	MAD	UAE	MAD	UAE	MAD	UAE	MAD
Growth	1	96 ± 3	93 ± 2	111 ± 7	115 ± 3	49 ± 5	47 ± 3	64 ± 3	65 ± 3
	2	24 ± 1	-	98 ± 5	-	21 ± 2	-	65 ± 3	-
	3	51 ± 6	-	78 ± 1	-	12 ± 2	-	62 ± 2	-
Fattening	1	36 ± 3	36 ± 1	158 ± 1	159 ± 2	51 ± 2	56 ± 6	71 ± 3	72 ± 2
	2	26 ± 1	-	126 ± 3	-	73 ± 2	-	72 ± 4	-
	3	3,9 ± 0,4	-	71 ± 3	-	38 ± 3	-	58 ± 2	-
Posture	1	53 ± 2	54 ± 1	110 ± 5	109 ± 1	47 ± 3	46 ± 3	62 ± 5	65 ± 2
	2	5,3 ± 0,3	-	96 ± 1	-	27 ± 3	-	61 ± 3	-
	3	4,7 ± 0,6	-	61 ± 3	-	14 ± 2	-	55 ± 3	-

UAE, Ultrasound-assisted extraction; MAD, Microwave-assisted digestion.

Three results from each sample for growth, fattening and posture using the proposed method were compared with the results of microwave-assisted digestion. Paired t-test was used to compare them with a 95% confidence level. Through this test, it was not noticed significant difference between data generated by the two methodologies. The t values found respectively for Cu, Mn, Ni and Zn were 0.555, 0.918, 0.305, and 2.50, all values below the tabled value (4.30) indicating that, at this confidence level, results found for both methods are not statistically different.

#### CONCLUSIONS

Application of mixture and Doehlert designs allowed the development of an analytical procedure for determination of copper, manganese, nickel and zinc from ration for chicken nutrition using flame atomic absorption spectrometry (FAAS), after ultrasonic-assisted extraction. Since FAAS is low cost and a relatively simple analytical technique, its association with efficient extraction procedures can make it more adequate for quality control in industries for chicken rations production.

The described method offers a quick, easy and efficient sample preparation method for direct determination of these metals from these samples. The use of ultrasonic energy allowed leaching of the target analytes in a shorter time than required by the conventional wet acid digestion. The ultrasonic-assisted extraction is a quick, inexpensive, easy, and precise technique.

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#### RESUMO

Neste trabalho, técnicas de otimização multivariada foram usadas no desenvolvimento de um método com base na extração assistida por ultrassom para determinação de cobre, manganês, níquel e zinco em rações para nutrição de frangos usando espectrometria de absorção atômica com chama. As proporções dos componentes extratores (soluções 2.0 mol.L<sup>-1</sup> dos ácidos nítrico, clorídrico e acético) foram otimizadas usando-se um planejamento de misturas centróide simplex. As proporções ótimas desta misturas representadas em percentagem de cada componente foram, respectivamente, 20, 37 e 43%. As variáveis de método (massa da amostra, tempo de sonicação e concentração final do ácido) foram otimizadas usando-se planejamento Doehlert. Os valores ótimos encontrados para estas três variáveis foram respectivamente 0,24g; 18s e 3,6 mol.L<sup>-1</sup>. O método desenvolvido permite a determinação de cobre, manganês, níquel e zinco com limites de quantificação de 2,82; 4,52; 10,7 e 9,69 µg.g<sup>-1</sup>, e precisão expressa como desvio padrão relativo (%SRD, 25 µg.g<sup>-1</sup>, N = 5) de 5,30; 2,13; 0,88 e 0,83%, respectivamente. Este método foi aplicado na determinação de analitos em rações de frango coletadas no comércio especializado da cidade de Jequié (Estado da Bahia/Brasil). A aplicação do test t

pareado aos resultados obtidos, no nível de confiança de 95%, mostrou não haver diferença significativa entre o método proposto e a digestão assistida por microondas.

**Palavras-chave:** rações para frango, extração assistida por ultrassom, planejamento de misturas, planejamento Doehlert, metais.

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