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Application of factorial design in optimization of preconcentration procedure for copper determination in soft drink by flame atomic absorption spectrometry

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Abstract

In the present paper, a procedure for preconcentration and determination of copper in soft drink using flame atomic absorption spectrometry (FAAS) is proposed, which is based on solid-phase extraction of copper(II) ions as its ion pair of 1,10-phenanthroline complexes with the anionic surfactant sodium dodecil sulphate (SDS), by Amberlite XAD-2 resin. The optimization process was carried out using 2^{4-1} factorial and 2^2 factorial with a center point designs. Four variables (XAD-2 mass, copper mass, sample flow rate and elution flow rate) were regarded as factors in the optimization. Student's *t*-test on the results of the 2^{4-1} factorial design with eight runs for copper extraction, demonstrated that the factors XAD-2 mass and sample flow rate in the levels studied are statistically significant. The 2^2 factorial with a center point design was applied in order to determine the optimum conditions for extraction. The procedure proposed allowed the determination of copper with detection limits ($3\alpha/S$) of 3.9 $\mu g\,l^{-1}$. The precision, calculated as relative standard deviation (R.S.D.) was 1.8% for 20.0 $\mu g\,l^{-1}$ of copper. The preconcentration factor was 100. The robustness of this procedure is demonstrated by the recovery achieved for determination of copper in the presence of several cations. This procedure was applied to the determination of copper in soft drink samples collected in Campinas, SP, Brazil.

Keywords: Copper; Factorial design; Flame atomic absorption spectrometry (FAAS); Soft drink

1. Introduction

Procedures for optimization of factors by multivariate techniques [1–5] have been encouraged, as they are faster, more economical and effective, and allow more than one variable to be optimized simultaneously. This optimization can be accomplished using experimental designs appropriate for determining first and second order models. The experimental designs not only determine the influence of the variables to be optimized for the response, but also enable the response function to be obtained and optimized.

In chemistry, the factorial design [6–15] has been widely used in several situations, such as: optimization of experimental variables in cyclic voltammetry of methylene blue [6]; optimization process for organic synthesis [7]; development of an chemiluminescent flow system for bromate determination [8]; methodology for reverse phase HPLC [9]; investigation of vibrational frequencies of methyl fluoride [10]; improving the Ti/TiO_2 electrodes performance [11]; optimization of an on-line preconcentration system for platinum determination [12]; determination of cadmium in urine specimens by graphite furnace atomic absorption spectrometry [13]; determination of phosphate in natural water employing a monosegmented flow system [14]; automatic on line preconcentration and determination of lead in water by ICP OES [15].

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Copper is an essential trace element for humans, stimulating the fundamental metabolic protein synthesis. Copper deficiency causes anemia, loss of weight and bone and cartilage with irregular physiological development. Abnormal ingestion causes neurological anomalies, hepatic and renal disturbances [16,17]. This metal is frequently determined in foods. However, their determination by FAAS is difficult because of its relatively low sensitivity and the high organic concentration [18]. According to the Brazilian legislation, copper concentration in water distributed for public provisioning is established in 20 $\mu g\,l^{-1}$ [19].

The reagent 1,10-phenanthroline (phen) forms complexes with several metal ions, including copper(II). 1,10-Phenanthroline have been used for different analytical determinations, such as: solid-phase extraction using silica gel [20,21] or tetraphenylborate-microcrystalline naphthalene adsorbent [22] and liquid–liquid extraction using several solvents [23,24];

In this work, a procedure for the preconcentration and determination of copper in soft drink using FAAS is proposed. Factorial designs were used for optimization of the experimental variables. It is based on the solid-phase extraction of copper ions as its ion pairs of 1,10-phenanthroline complexes with the anionic surfactant sodium dodecyl sulphate on Amberlite XAD-2 resin (polystyrene–divinylbenzene polymer).

2. Experimental

2.1. Instrumentation

A Perkin-Elmer Model 5100PC flame atomic absorption spectrophotometer was used for copper determination. The absorption measurements were made under conditions described in Table 1. The calibration curves (0–4.0 $\mu g\,ml^{-1}$) for copper were established with solutions prepared from a 1000 $\mu g\,ml^{-1}$ stock solution. A Procyon pH meter (PHD-10 model) was used to pH values measurements.

2.2. Reagents

All the reagents were of analytical reagent grade. Deionized-distilled water (DDW) was used throughout the experimental work. Laboratory glassware was kept overnight in 10% (v/v) nitric acid solution, and washed with deionized water before use.

Table 1
Operating parameters for flame atomic absorption spectrophotometer

Wavelength (mm)	324.8
Lamp current (mA)	15
Slit width (nm)	0.7
Acetylene flow rate ($l\,min^{-1}$)	2.0
Air flow rate ($l\,min^{-1}$)	10.0
Sample flow rate ($ml\,min^{-1}$)	10.0

The copper stock solution (1000 $\mu g\,ml^{-1}$) was prepared by dissolving the metal (Baker 99.96%) in 5 ml of concentrated nitric acid and diluting with DDW to 1000 ml.

Phen solution ($1.7 \times 10^{-2}\,mol\,l^{-1}$) was prepared by dissolving 0.1680 g of 1,10-phenanthroline (Merck) in ethanol (1 ml) and diluting with DDW to 50 ml.

SDS solution ($1.7 \times 10^{-2}\,mol\,l^{-1}$) was prepared by dissolving 0.4900 g of sodium dodecylsulphate (Merck) in 100 ml of DDW.

Acetate buffer solution (pH 5.0) was prepared by mixing 52.5 g of anhydrous sodium acetate and 21.2 ml of concentrated acetic acid and diluted to 1 l of DDW.

Amberlite XAD-2 (Aldrich) was treated with hydrochloric acid 2.8 $mol\,l^{-1}$ for 60 min. Afterwards the resin was washed with deionized water until neutral pH, and then was washed with methanol, DDW and ethanol. Finally, it was dried in an oven at 85 °C for 35 min. The packing of the column was done using a conditioning solution prepared with 2 ml of the buffer solution in 50 ml of DDW.

2.3. Samples

Reference material supplied by Instituto de Pesquisas Tecnológicas do Estado de São Paulo, Brazil (steel IPT-97) was analysed. Composition: C = 0.165%; Si = 0.231%; Mn = 1.11%; P = 0.015%; S = 0.026%; Cu = 0.129%; Ni = 0.227%; Cr = 1.22%; Mo = 0.064%; Al = 0.028%; Co = 0.012%; V = 0.024%; Nb = 0.023%; N = 0.0119%; Ti = 0.002%; B = 0.0022%. The procedure for chemical decomposition is described as follows: 0.1071 g of the sample was treated in a 50 ml beaker with 20 ml of 37% hydrochloric acid/65% nitric acid [3:1, v/v]. After heating to dryness the residue was then treated with 25 ml of 7 $mol\,l^{-1}$ hydrochloric acid. The obtained solution was extract with 25 ml methyl–isobutyl ketone for iron separation [25,26]. The aqueous phase was heated once again, until dry. The residue was taken with 1 ml of concentrated HNO_3 and the volume was completed to 1 l. This solution was used in the preconcentration step, in order to simulate a synthetic water sample with common metals found in soft drinks at $\mu g\,l^{-1}$ levels, since reference materials for soft drinks are not commercially available.

Soft drink samples were collected in Campinas, SP, Brazil and were mechanically stirred for 6 h to degas. The samples were analyzed directly and after addition of copper to perform a recovery test.

2.4. General procedure

It was transferred 1 ml of sample solution contained copper ions (10 $\mu g\,ml^{-1}$) into a 150 ml becker and 2 ml of buffer solution pH 5.0 was added, followed by 1 ml of 1,10-phenanthroline solution ($1.7 \times 10^{-2}\,mol\,l^{-1}$), 1 ml of SDS solution ($1.7 \times 10^{-2}\,mol\,l^{-1}$). Final concentration of copper in solution is of 1 $\mu g\,ml^{-1}$. This solution was passed through

Table 2
Factors and levels used in the factorial design for extraction of copper

Variable	Low (−)	High (+)
XAD-2 mass (mg)	70	140
Copper mass (μg)	100	200
sample flow rate (ml min^{-1})	1	4
Elution flow rate (ml min^{-1})	0.7	1.5

the column loaded with Amberlite XAD-2 resin, and in the sequence the resin was washed with 10 ml of DDW. The adsorbed copper(II) ions as its ion pair of 1,10-phenanthroline complexes on the column was then eluted with ethanol. The eluent was directly collected in a 5 ml volumetric flask and the volume completed with DDW. This solution was used for the determination of copper by FAAS technique using the resonance line 324.8 nm.

2.5. Procedure used in the factorial design

The general procedure was applied using the variable experimental conditions for XAD-2 mass, copper mass, sample flow rate and elution flow rate showed in Table 2. Maximum and minimum levels of each factor were chosen according to data from previous experiments. The experimental data were processed using the freeware FACTORIAL program [27].

2.6. Optimization strategy

The optimization process was carried out using a factorial design 2^2 with center point. The statistically significant variables (XAD-2 mass and sample flow rate) were regarded as factors, and the experimental data were processed using the freeware MODREG program [28].

3. Results and discussion

3.1. Screening analysis of the system

The proposed procedure is based on the solid-phase extraction of copper(II) ions as its ion pair of 1,10-phenanthroline complexes with the anionic surfactant sodium dodecyl sulphate using Amberlite XAD-2 resin. The following factors were evaluated: XAD-2 mass, copper mass, sample flow rate and elution flow rate. A two-level fractionary factorial design of four factors (2^{4-1}) in duplicate was used in a preliminary study in order to detect the main factors of the extraction process. Table 2 lists the maximum and minimum values given to each factor and Table 3 shows the experimental design matrix and the results derived from each run in duplicate for copper. The significance of the effects was evaluated with the *t*-test (*t* of Student). These are presented in Table 4. The *t*-values are calculated dividing the effect values by the standard error of 1.225. It is considered that *t*-values above the tabulated $t_{8.95\%} = 2.306$ are significant at the 95% confidence level.

Table 3
Design matrix and the results of copper extraction

Runs	XAD-2 mass	Copper mass	Sample flow rate	Elution flow rate	Copper extraction (%)
1	–	–	–	–	80/82
2	+	–	–	+	102/101
3	–	+	–	+	79/80
4	+	+	–	–	101/99
5	–	–	+	+	59/61
6	+	–	+	–	75/71
7	–	+	+	–	57/60
8	+	+	+	+	77/74

The Table 4 interpretation demonstrates that the factors XAD-2 mass and sample flow rate are highly significant. An increase in XAD-2 mass increases the extraction efficiency and an increase in sample flow rate decreases the extraction efficiency. Such a result is consistent considering that the sorbent mass and percolating flow rate are related to the time of contact in a dynamic process [29].

3.2. Optimization of the system

With the results showed in Table 3 it can be observed that there exist a region with maximum percentage of extraction (99–102%), however such percentage was obtained with low flow rates (1 ml min^{-1}). With the objective of improving the speed process to work with larger volumes of sample, it was necessary to optimize the analytical parameters involved. The variables chosen for the optimization were mass of sorbent and sample flow rate. In the optimization of the process a factorial design 2^2 with center point containing three levels for each variable was performed. Table 5 shows the design matrix and the results obtained.

With the obtained results and the use of the mathematical modeling using MODREG program [28], the response surface for this system was determined. The adjusted response

Table 4
Principal and interaction effect values for the 2^{4-1} factorial

	E (%)	*t*-values
Average	78.6	
Principal effects		
1	17.8	14.53[a]
2	−0.5	−0.41
3	−23.7	−19.35[a]
4	1.0	0.82
Interactions of two factors		
12	1.0	0.82
13	−2.8	−2.29
14	1.0	0.82
23	1.0	0.82
24	2.8	2.29
34	1.0	1.0

[a] Significant *t*-values at the 95% confidence level, *t* = effect/S.E.

Table 5
Design matrix and the results of copper extraction

Runs	XAD-2 mass	Sample flow rate	X_1	X_2	E (%)
1	70	3	−1	−1	66
2	210	3	+1	−1	100
3	70	5	−1	+1	54
4	210	5	+1	+1	92
5	140	4	0	0	75
6	140	4	0	0	71
7	140	4	0	0	77
8	140	4	0	0	74

The codified variables are: $X_1 = (m - 140/70)$ (II) $X_2 = (V - 4/1)$ (III) m is the XAD-2 mass and V is the sample flow rate.

was a first degree equation, as described by Eq. (1).

$$y = 76.13 + 18x_1 - 5x_2 \quad (\pm 0.88)(\pm 1.25)(\pm 1.25) \tag{1}$$

The relatively small values for the errors of the coefficients of the equation, showed in parenthesis below each term, indicates that the linear model is significant. Curvature and interaction effects were not found to be significant at the 95% confidence level indicating that the model does not have significant lack of fit. Fig. 1 shows the response surface as function of the two most significant factors, the mass of the sorbent and the sample flow rate.

Table 6
Copper liquid–solid extraction with the XAD-2 mass calculated from Eq. (1)

Test	XAD-2 (mg)	Flow rate (ml min^{-1})	Cu (II) (μg)	E (%)
1[a]	235	3.7 ± 0.1	100	>98
2[b]2	935	35.0 ± 5.0	20	>98

[a] [Cu(II)] = 1 μg ml^{-1}.
[b] [Cu(II)] = 26.6 μg l^{-1}.

3.3. Response characteristics of the optimized system

Using Eq. (1), the mass of XAD-2 necessary to reach quantitative extractions was calculated using a sample flow rates of 3.7 ml min^{-1} (low rate) and 35.0 ml min^{-1} (high rate). So, the calculated XAD-2 masses were 235 and 935 mg (dry weight), respectively. These conditions were used to evaluate the developed model, furnishing the results showed in Table 6. After optimization, the proposed procedure was applied to the determination of Cu(II) in aqueous solution and Table 6 shows the results.

The optimization of the liquid–solid extraction process was done with maximum sample flow rate of the 5 ml min^{-1}. For test *2 included in Table 6, the sample flow rate was 35.0 ml min^{-1} with a XAD-2 mass of 935 mg. Nevertheless, high flow rates contributes to decreaseing extraction (given the negative signal of x_2), and it is more than compensated for by the positive mass contribution (positive signal of x_1). The model has a gradient mass ($18x_1$) more than three times

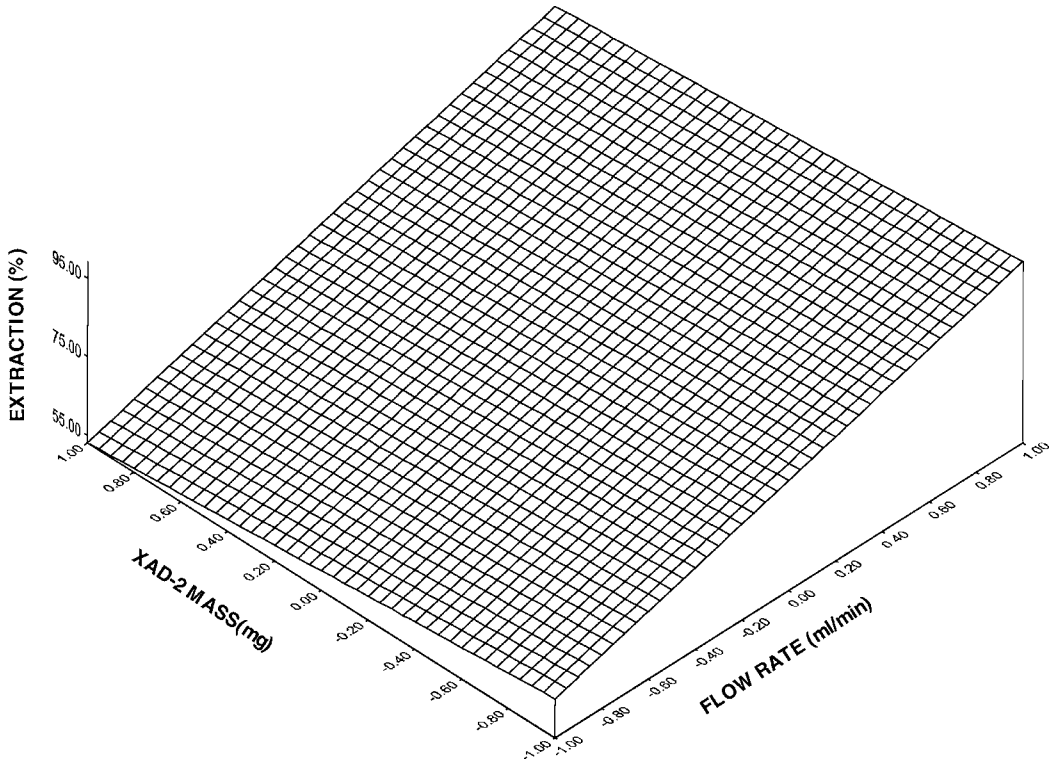

Fig. 1. Response surface for the copper extraction as function of the sorbent mass and sample flow rate.

larger than the absolute value of the flow rate gradient ($5x_2$). This shows that the model is robust even outside the tested intervals.

3.4. Analytical features

The precision calculated as the relative standard deviation (R.S.D.) for a series of nine replicates was 1.8% for the level of 20.0 μg l^{-1} of copper in natural waters.

The preconcentration factor was 100, considering the aqueous sample volume of 1000 ml and a solution volume for analysis of 10.0 ml.

The limits of detection (LOD) and quantification (LOQ), defined as LOD = $(3\alpha)/S$ and LOQ = $(10\alpha)/S$, where S is the slope of the analytical curve and α is the standard deviation of 10 consecutive measurements of the blank, were 3.9 and 12.8 μg l^{-1}, respectively [30].

3.5. Effect of other metal ions on the proposed procedure

Analytical FAAS is a well established and very specific technique and low sensitive to interferences. Then, the potential interferences effects occurring in this procedure are mainly related to the extraction during the preconcentration step applied to the target samples, and this effect should be particularly relevant with respect to the consumption of the analytical reagent. Considering the samples of interest, and the most probable metal ions reported as being regularly present, Fe, Mn and Al were evaluated as the most problematic interfering elements in the extraction step, because they form stable complexes with *o*-phenanthroline.

In order to verify the effect of other metal ions on the proposed method, copper (20.0 μg) was determined in the presence of 80.0 μg of iron, manganese and aluminum in 600 ml of aqueous solution. The copper recovery was better than 98%.

3.6. Accuracy

In order to evaluate the accuracy of the proposed procedure developed, copper was determined in the aqueous sample solution prepared from the steel IPT-97 reference material. The result achieved was 40.7 ± 0.7 μg ml^{-1} compared to the expected value of 41.7 μg ml^{-1}, according to the original amount of copper in the certified steel. This test was carried out using 300.10 g of the prepared solution in triplicate.

3.7. Analytical application

The optimized methodology was applied to the analysis of soft drink samples (carbonated lemon and guaraná diet) collected in supermarkets of Campinas, SP, Brazil, with and without copper spikes. A volume of 600 ml of degassed soft drink sample was used for analysis and this was spiked with 30 or 12 μg of copper. The results are shown in Table 7 including the recovery data for added copper. The copper levels found in this study from soft drink samples were 100 times lower than obtained for carbonated colas [31]. The recovery of copper added to the samples before application of the method proposed demonstrates its efficiency.

Table 7
Determination of Cu (II) in soft drinks

Sample	Mass of added copper (μg)	Copper found (μg)[a]	Recovery of copper (%)
1[b]	0	2.7 ± 0.1	99
	30	32.6 ± 0.9	
2[c]	0	2.1 ± 0.2	98
	12	13.9 ± 0.6	
3[b]	0	2.4 ± 0.2	106
	12	15.1 ± 0.5	

[a] At 95% confidence level.
[b] Carbonated lemon diet.
[c] Carbonated guaraná diet.

4. Conclusion

Application of factorial designs allowed the optimization of a procedure for the determination of copper by FAAS, based on solid-phase extraction, using a smaller number of experiments. The robustness of the obtained model allows to use flow rates as high as 35 ml min^{-1}.

The obtained limits of detection and quantification, allow the determination of copper under the maximum tolerable value prescribed by the Brazilian legislation.

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References

[1] B.B. Neto, I.S. Scarminio, R.E. Bruns, Como fazer experimentos, Editora da Unicamp, Campinas, SP, 2001.
[2] G.E.P. Box, W.G. Hunter, J.S. Hunter, Statistics for Experimenters. An Introduction to Design, in: Data Analysis and Model Building, Wiley, New York, 1978.
[3] D.C. Montgomery, Design, Analysis of Experiments, Wiley, Singapore, 1984.
[4] S.L.C. Ferreira, M.A. Bezerra, W.N.L. dos Santos, B.B. Neto, Talanta 61 (2003) 295.
[5] W.N.L. dos Santos, C.M.N. dos Santos, S.L.C. Ferreira, Microchem. J. 75 (2003) 211.
[6] R.F. Rocha, S.S. Rosatto, R.E. Bruns, L.T. Kubota, J. Electroanal. Chem. 433 (1997) 73.
[7] J. Guervenou, P. Giamarchi, C. CouLOuarn, M. Guerda, C. Le Lez, T. Oboyet, Chemom. Intell. Lab. Syst. 63 (2002) 81.
[8] J.C.G.E. da Silva, J.R.M. Dias, J.M.C.S. Magalhães, Anal. Chim. Acta 450 (2001) 175.

[9] R.L.V. Ribeiro, C.B. Grespan, C.H. Collins, K.E. Collins, R.E. Bruns, J. High Resol. Chromatogr. 22 (1999) 52.
[10] A.L.M.S. Andrade, B.B. Neto, W. Souza, G.M. Campos-Takaki, J. Comp. Chem. 17 (1996) 167.
[11] C.M. Ronconi, E.C. Pereira, J. Appl. Electrochem. 31 (2001) 319.
[12] S. Cerutti, J.A. Salonia, S.L.C. Ferreira, R.A. Olsina, L.D. Martinez, Talanta 63 (2004) 1077.
[13] E.A.H. Caraballo, M. Burguera, J.L. Burguera, Talanta 63 (2004) 419.
[14] M.C.T. Diniz, O.F. Filho, E.V. Aquino, J.J.R. Rohwedder, Talanta 62 (2004) 469.
[15] M. Zougagh, A.G. Torres, E.V. Alonso, J.M.C. Pavón, Talanta 62 (2004) 503.
[16] E.J. Underwood, Trace Elements in Human and Animal Nutrition, Academic Press, New York, 1977.
[17] D.L. Tsalev, Z.K. Zaprianov, Atomic Absorption Spectrometry in Occupational and Environmental Health Practice, CRC Press, Boca Raton, Florida, 1984.
[18] B. Welz, M. Sperling, Atomic Absorption Spectrometry, Wiley-VCH, Weinheim, 1999.
[19] http://www.mma.gov.br/part/CONAMA/res/resol86/re2086.htm, Resolução CONAMA no 20 de 18 de Junho de 1986 (accessed in 12 September 2003).
[20] A. Ali, X. Yin, H. Shen, Y. Ye, X. Gu, Anal. Chim. Acta 392 (1999) 283.
[21] O. Zaporozhets, O. Gawer, V. Sukhan, Colloids Surf. A 147 (1999) 273.
[22] M.A. Taher, B.K. Puri, Indian J. Chem. A 42 (2003) 2963.
[23] C.G. Taylor, B. Fryer, Analyst 94 (1969) 1106.
[24] T. Koh, T. Sugimoto, Anal. Chim. Acta 333 (1996) 167.
[25] G.H. Morrison, H. Freiser, Solvent Extraction in Analytical Chemistry, Wiley, New York, 1957.
[26] M.G.A. Korn, H.V. Jaeger, A.C. Ferreira, A.C.S. Costa, Spectrosc. Lett. 33 (2000) 127.
[27] R.E. Bruns, I.S. Scarminio, B.B. Neto, FACTORIAL—Planejamentos Fatoriais, http://www.chemkeys.com/bra/md/peeo_6/tepc_2/tepc_2.htm (accessed in 12 September 2003).
[28] R.E. Bruns, I.S. Scarminio, B.B. Neto, MODREG—Modelagem por mínimos quadrados, http://www.chemkeys.com/bra/md/peeo_6/tepc_2/tepc_2.htm (accessed in 12 September 2003).
[29] R. Ciola, Fundamentos da Catálise, Editora Moderna, São Paulo, 1981.
[30] M. Thompson, H.M. Bee, R.V. Cheeseman, W.H. Evans, D.W. Lord, B.D. Ripley, R. Wood, Analyst 112 (1987) 199.
[31] P.C. Onianwa, I.G. Adetola, C.M.A. Iwegbue, M.F. Ojo, O.O. Tella, Food Chem. 66 (1999) 275.