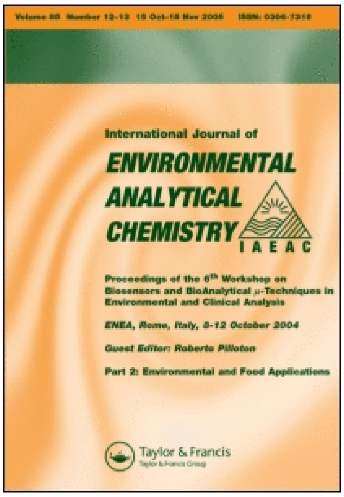

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Flow injection determination of copper and iron in seafoods by a continuous ultrasound-assisted extraction system coupled to FAAS

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Flow injection determination of copper and iron in seafoods by a continuous ultrasound-assisted extraction system coupled to FAAS

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Copper and iron were extracted on-line from solid seafood samples by a robust, fast and simple continuous ultrasound-assisted extraction system (CUES). CUES is connected to a flow-injection manifold, which allows on-line flame atomic absorption spectrometric determination of copper and iron. Experimental designs were used to optimize the continuous leaching procedures. These methods allowed a total sampling frequency of 46 and 18 samples per hour, with relative standard deviations of 1.6% and 0.3%, for copper and iron, respectively (for a sample containing 13.6 μg/g of copper and 217.3 μg/g of iron (dry mass)). The limits of detection for 30 mg of sample were 0.3 μg/g for copper and 0.6 μg/g for iron (dry mass). Analytical procedures were verified by the analysis of a standard reference material (lobster hepatopancreas marine, TORT-1) and were applied to several real seafood samples from the estuaries of Galicia (Spain) with satisfactory results.

Keywords: Continuous ultrasound-assisted extraction system; Flame atomic absorption spectrometry; Copper determination; Iron determination; Seafood samples

1. Introduction

Copper and iron are closely implicated in metabolic processes of the human metabolism. In addition to their nutritional interest, the determination of these elements in seafood samples contributes to obtain environmental information [1]. Seafoods are important biological monitors because the metal concentrations found in them are closely related to the degree of environmental contamination of the surrounding environment [2].

Several methodologies have been proposed for the determination of trace metals in solid samples, above all involving electrothermal atomic absorption spectrometry (ETAAS) and flame atomic absorption spectrometry (FAAS) detectors [3–8]. Nevertheless, the preparation of a solid sample is often the most problematic analytical

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step because it is time-consuming and presents several potential drawbacks. Most solid-sample pretreatment procedures are still performed manually, making them slow, complex, tedious and usually the source of analytical errors (random errors are mainly associated with glassware calibration) and contamination problems [9]. Automation of the solid sample pretreatment in the analytical process is an arduous task. In this sense, flow-injection (FI) techniques have been shown to be very useful in automating solid-sample pre-treatment [10]. Generally, when continuous flow techniques are used for the pretreatment of solid samples, external energy, such as ultrasonic or microwave energy, is utilized. The insertion of a microwave oven into an FI manifold offers many advantages over off-line digestion methodologies: it enables automated operations, minimizes extensively the time delay between sample delivery and analysis, allows easy digestion of complex matrices, improves personal safety, and decreases any losses of volatile analytes [11,12]. Nevertheless, these advantages are accompanied by certain drawbacks such as matrix interference, since the sample matrix is introduced into the detector, and a cooling area in the FI system is needed due to the high temperature achieved in the oven. Ultrasound has emerged as an alternative method for solid-sample pre-treatment and is used in quantitative extraction of metal ions using diluted acids [13–15]. FI-ultrasound assisted extraction has several advantages over its off-line counterparts, such as minimization of sonication time (reduced by a factor of 6–12) and reagent consumption. Furthermore, the centrifugation step to separate the liquid phase (which usually requires 10–20 min) is removed, thus simplifying the process considerably [16–17].

In this paper, a continuous ultrasound extraction system (CUES) incorporated into an on-line flow injection manifold is described and optimized for copper and iron determination in seafood samples.

2. Experimental

2.1. *Instrumentation*

The overall manifold consists of the following: (A) the CUES, which carries out the sample lixiviation; and (B) copper or iron analysis by FAAS (figure 1). The CUES is composed of a Gilson Minipuls-3 peristaltic pump fitted with PTFE tubes, an ultrasonic bath (Selecta), a glass minicolumn (50 mm × 3 mm i.d., bed volume 350 μL) (Omnifit) used as a sample container (the ends of the minicolumn were plugged with filter paper (Whatman 541)) and two Reodyne (models 5041 and 5020) low-pressure valves. The on-line copper or iron monitoring was performed by a manifold comprising a Gilson Minipuls-3 peristaltic pump fitted with PTFE tubes and two Reodyne (model 5020 and 5301) injection or switching valves. PTFE tubes (0.8 mm i.d.) were used to connect the FI manifold to a Perkin Elmer 5000 atomic absorption spectrometer with deuterium background correction. This analytical instrument was furnished with a suitable hollow-cathode lamp and set at 324.8 or 248.3 nm, for copper or iron, respectively. A standard air/acetylene flame was used. The spectrometer output was connected to a Perkin Elmer 50 servograph recorder with a range of 5 mV. The signals measured were the heights of the absorbance peaks. Numerical analyses of experimental designs were performed by means of the Statgraphic V.4.1 statistical package (Manugistic, Rockville, MD).

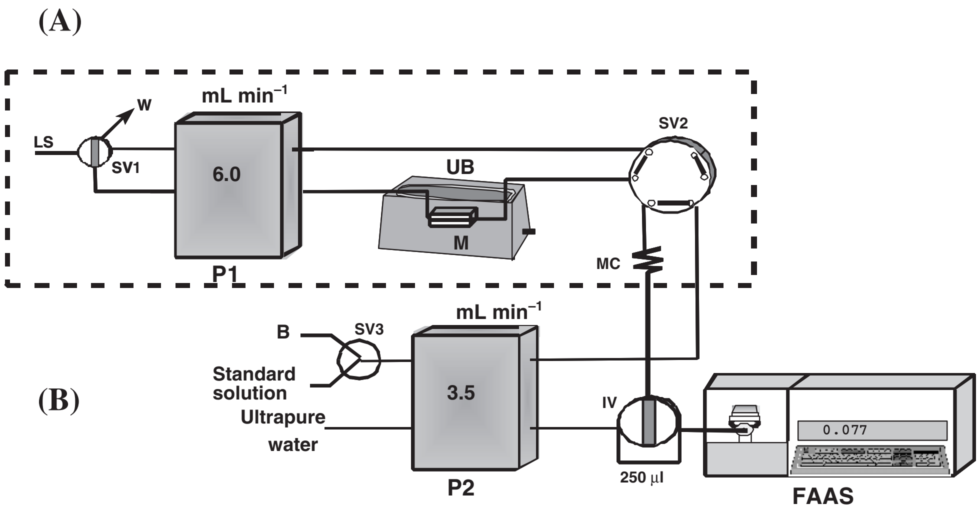

Figure 1. Schematic diagram of the CUES and optimum working conditions for the on-line Cu and Fe determination: (A) on-line acid leaching step; and (B) on-line Cu/Fe monitoring. P1 and P2: peristaltic pumps; UB: ultrasonic bath; M: minicolumn; IV: injection valve; SV: switching valves; MC: mixing coil; W: waste; B: blank and FAAS. flame atomic absorption spectrometer.

2.2. *Material, reagents and solutions*

Ultrapure water of 18 MΩ cm resistivity obtained from Milli-Q water purification system (Millipore) was used for the preparation of samples, reagents and standards. The glassware used was cleaned in 4 M HNO_3 for 4 days and rinsed with ultrapure water before use. Hydrochloric acid, nitric acid, copper (1000 µg/mL) and iron (1000 µg/mL) standards were of reagent grade (Merck). The certified reference material used to validate the proposed method was TORT-1 (lobster hepatopancreas marine, National Research Council of Canada) with a certified concentration of 439 ± 22µg/g for Cu and 186 ± 11 µg/g for Fe.

2.3. *Sample preparation*

Fresh mussel and clam samples were collected from Galician coast (Northwest Spain). Other seafoods were purchased in local markets. The samples were triturated, ground, blended, homogenized, freeze-dried, and then kept in clean, dry containers. In all cases, after sieving, fractions with particle size under 100 µm were taken.

2.4. *Procedure for copper and iron determination in seafood samples*

The continuous copper and iron determination system is shown in figure 1. Seafood samples (30 mg) were directly weighted into a glass minicolumn. Then, the minicolumn was connected to the CUES. First, the CUES circuit (1 mL) was loaded on-line with the acid leaching solution (3 M nitric acid). Then, the SV1 was switched to its other position, thereby closing the CUES circuit. The leaching solution was circulated at 6.0 mL/min under ultrasonic irradiation through the minicolumn for 1 min for copper and 3 min for iron extraction, changing the direction of the flow each 20 s to prevent sample accumulation at the minicolumn ends. Then, the SV2 was switched to its other position, the acid extract circulated towards the mixing coil to be homogenized, and finally, 250 µL of this acid extract was injected by means of an IV into an ultrapure water carrier stream transporting it to the detector. After the sample measurement was accomplished, the CUES circuit was washed with ultrapure water to avoid carryover, and a new minicolumn was inserted into the closed circuit for the next analysis. Standard solutions containing 0–5 µg/mL of copper or iron, in the same acid medium as the leaching solution, were injected into an ultrapure water carrier stream by IV. Blank determinations were done using the acid leaching solution, and in all instances the absorbance was close to 0.000.

For routine analysis, 20 minicolumns were sufficient for several analytical cycles in a working day. The time required to clean up and dry these type of minicolumns was about 2 min.

3. Results and discussion

Optimization of the whole proposed procedure focused on the continuous ultrasound extraction step. The detection step was optimized taking into account the optimum values found for the CUES.

To study the behaviour of the variables involving the CUES (nitric acid concentration, hydrochloric acid concentration, sonication time, leaching temperature, flow rate of the CUES and leaching volume), a factorial Plackett-Burman 2^6*3/16 type III resolution design allowing 5 degrees of freedom plus one centre point was built. The lower and upper values assigned to each variable were chosen from the available data and experience from previous experiments (table 1). This experimental design involves 13 non-randomised runs. To test the statistical significance of the effects, an ANOVA (from Statgraphic V. 4.1) was used. This factorial design has been applied to a certified reference material (10 mg, TORT-1). To optimize the CUES, copper and iron were measured on-line in the leachate by FAAS with a flow system similar to that depicted in figure 1. The variable response was %Cu and %Fe recovery. The numerical analysis of the results produced the standardized main effects Pareto Chart (figure 2). In this chart, the bar lengths are proportional to the absolute value of the estimated effects, to help compare their relative importance. The conclusion

Table 1. Factor levels in the Plackett Burman factorial designs and their optimum values for copper and iron determination.

Factor	Key	Low	High	Optimum for copper determination	Optimum for iron determination
HNO_3 concentration (M)	A	0	3	3	3
HCl concentration (M)	B	0	3	0	0
Sonication time (min)	C	0.5	5	1	3
Leaching temperature (°C)	D	20	70	20	20
Flow rate of the CUES (mL/min)	E	3.5	6	6	6
Leaching solution volume (mL)	F	1	3	1	1

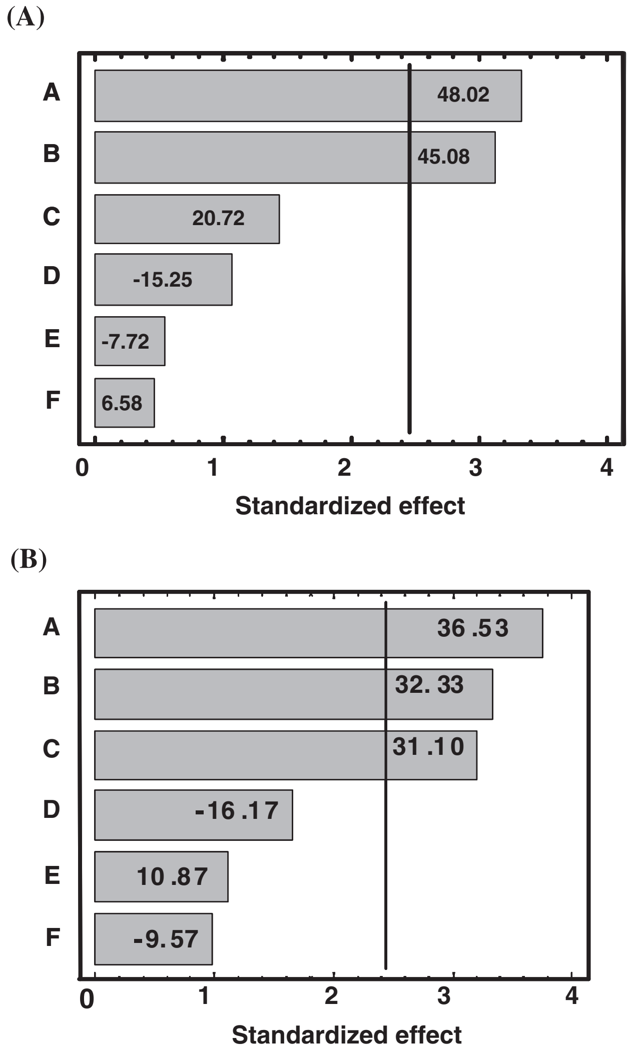

Figure 2. Standarized Pareto charts for the Plackett Burman design 2ˆ6*3/16 for the continuous ultrasonic acid extraction of (A) copper and (B) iron from seafood samples. The vertical line indicates the statistical significance ($P = 95\%$) bound for the effects.

from the copper extraction screening study is that the concentrations of nitric and hydrochloric acids are influential positive factors between the ranges studied because these factors overtake the limit of statistical significance (95%). With regard to the non-significant variables, sonication time and leaching volume present, a positive estimated effect and leaching temperature and flow rate of the CUES have a negative effect. The iron-extraction screening study reflected three factors of statistical significance (concentration of nitric and hydrochloric acids, and sonication time with a positive estimated effect). A non-significant variable, such as leaching volume, presents a positive estimated effect, while leaching temperature and flow rate of the CUES have a negative effect. Since the Plackett-Burman design only provides the tendencies rather than optimum values, the variables were fine-tuned outside the framework of the design. In the case of concentrated acids (leaching solution), the aim was to decrease these concentrations because high acid concentrations can produce serious damage in the spectrometer nebulizer of FAAS. In this sense, several experiments were carried out, demonstrating that a nitric acid concentration of 3 M (without hydrochloric acid) as leaching solution was sufficient to obtain quantitative copper and iron recoveries (99.3% and 96.1%, respectively). With regard to the flow rate of the CUES and sonication time, they were also fine-tuned outside the framework of the design to achieve the highest sampling frequency for each determination. In this way, the sonication time was studied at a flow rate of 3.5 and 6.0 mL/min for both trace metals. It was observed that the minimum sonication time to obtain a quantitative recovery was 1 min for copper and 3 min for iron at a flow rate of 6 mL/min. Thus, these values were chosen as an optimum for these variables. Quantitative recoveries from leaching processes were achieved at room temperature; in addition, this optimum value simplified the FI system because there was no need for temperature control. It is important to diminish the value of the leaching volume to increase the sensitivity of the method. The experimental results showed that 1.0 mL of leaching solution is sufficient to extract quantitatively the copper and the iron present in the sample.

There are two additional variables that can affect the acid-extraction process: seafood particle size and sample amount. These variables were studied using the optimum conditions established for the CUES. The sample particle size was studied using sizes smaller than 30 µm and between 30 and 100 µm. The results obtained showed that this variable does not affect the extraction process, which can be explained as a result of the high energy supplied by the ultrasound energy (frequency of 40 kHz) that increases the contact between the sample and the leaching solution. In terms of the amount of sample, it was found that sample amounts greater than 30 mg generated pressures inside the CUES. Other flow parameters involving copper and iron determination were also optimized. The mixing coil length was fixed to 200 cm (equivalent to 1 mL); a shorter length does not lead to total homogenization of the extract, and a longer length increases the analysis time too much, thus reducing the sampling frequency. The carrier flow rate and injected volume were also studied. The carrier flow rate was studied between 3.0 and 6.0 mL/min. The aspiration flow rate of the nebulizer was adjusted to be the same as the flow rate of the carrier solution. Although the higher aspiration flow rate provided a better sensitivity, at the same time a higher degree of dispersion took place because the carrier flow rate was increasing. To obtain the minimum dispersion in the flow system, the optimum carrier flow rate was 3.5 mL/min (dispersion equal to 1.1). In relation to the injected acid extract volume, 250 µL was chosen as the optimum because this volume allowed two acid-extract injections for each sample, thus confirming its homogeneity.

4. Analytical figures of merit

The calibration graphs ($n = 8$) were run under the optimal chemical and flow conditions for the whole process. The equations for copper and iron were, respectively, absorbance $= 6.7 \times 10^{-4} + 0.046\,[\mathrm{Cu}]([\mathrm{Cu}] = 0\text{–}5\ \mu\mathrm{g/mL})$ and absorbance $= 4.8 \times 10^{-4} + 0.030\,[\mathrm{Fe}]$ ($[\mathrm{Fe}] = 0\text{–}5\ \mu\mathrm{g/mL}$).

Analytical procedures were verified by the analysis of a standard reference material (10 mg of TORT-1, lobster hepatopancreas marine). The Cu and Fe concentrations obtained (mean ± SD, $n = 3$) were 438.7 ± 1.3 µg/g and 186.6 ± 1.9 µg/g, respectively, which agrees with the certified values.

The precision of the continuous analytical method obtained for real samples was checked using a sample containing 13.6 ± 0.7 µg/g of Cu and 217.3 ± 1.1 µg/g of Fe (dry mass). The results expressed as relative standard deviation were 1.6% and 0.3% ($n = 11$) for Cu and Fe, respectively. The limits of detection (LOD) based on three times the standard deviation of the blank ($n = 30$) were 0.3 µg/g for copper and 0.6 µg/g for iron (dry mass) for 30 mg of sample. The sample throughputs, taking into account the whole process, were 46 and 18 samples per hour for copper and iron determination, respectively.

5. Analysis of samples

The method was used to determine copper and iron in several seafood samples (table 2). The results obtained by the proposed method were compared with those achieved by a conventional off-line sample digestion method using concentrated nitric acid with subsequent copper and iron determination by FAAS. The paired *t*-test [18] was used to compare the results obtained by both methods; it was concluded that neither method gives significantly different values for the copper and iron concentration, and thus, the agreement between the two methods is satisfactory. The results obtained were also analysed by hierarchical cluster analysis. This analysis was used, searching

Table 2. Determination of Cu and Fe in seafoods and paired *t*-test (mean ± S.D., $n = 3$).

	Cu (µg/g dry mass)		Fe (µg/g dry mass)	
Sample	Reference method[a]	Present method	Reference method[a]	Present method
Mussel 1	13.6 ± 0.7	13.8 ± 0.5	255.9 ± 1.9	255.1 ± 1.0
Mussel 2	12.9 ± 0.7	12.8 ± 0.5	257.8 ± 1.9	256.2 ± 1.0
Mussel 3	13.4 ± 0.4	13.1 ± 0.5	218.5 ± 1.1	217.0 ± 1.0
Mussel 4	10.9 ± 0.7	11.3 ± 0.5	221.1 ± 1.1	221.2 ± 1.0
Mussel 5	13.2 ± 0.4	13.1 ± 0.5	227.4 ± 1.9	227.7 ± 1.0
Mussel 6	11.2 ± 0.4	11.6 ± 0.0	242.6 ± 1.9	240.2 ± 1.0
Mussel 7	16.2 ± 0.7	16.0 ± 0.5	254.6 ± 1.1	255.6 ± 0.0
Mussel 8	18.2 ± 0.7	18.8 ± 0.5	246.4 ± 1.9	246.1 ± 1.0
Mussel 9	13.6 ± 0.7	13.1 ± 0.5	229.3 ± 1.9	229.5 ± 1.0
Mussel 10	14.0 ± 0.4	14.4 ± 0.0	248.3 ± 1.9	248.5 ± 0.0
Tuna	10.9 ± 0.7	11.3 ± 0.5	64.2 ± 1.9	65.7 ± 1.0
Sardine	12.3 ± 0.7	12.2 ± 0.5	94.0 ± 1.1	94.7 ± 1.0
Hake	5.9 ± 0.4	5.9 ± 0.0	51.6 ± 1.1	51.4 ± 1.0
Crab	6.3 ± 0.7	6.3 ± 0.5	37.0 ± 1.1	37.2 ± 1.0
Prawn	9.0 ± 0.7	9.4 ± 0.5	144.6 ± 1.1	144.0 ± 1.0
Razor-shell	7.0 ± 0.7	6.9 ± 0.0	59.8 ± 1.1	59.7 ± 0.0
Scallop	10.3 ± 0.7	10.3 ± 0.5	136.3 ± 1.9	136.9 ± 1.0
Cockle	8.3 ± 0.7	8.4 ± 0.5	115.5 ± 1.9	116.1 ± 1.0
Clam 1	10.9 ± 0.7	10.9 ± 0.5	219.8 ± 1.9	220.0 ± 0.0
Clam 2	12.9 ± 0.7	13.1 ± 0.5	211.6 ± 2.9	212.3 ± 1.0

[a] Off-line acid digestion and FAAS determination. Critical value of $t = 2.09$. For Cu: X (mean difference): -8.300×10^{-2}; S.D.: 0.263; $n = 20$; $t = 1.41$. For Fe: X (mean difference): -2.350×10^{-2}; S.D.: 0.907; $n = 20$; $t = 0.12$.

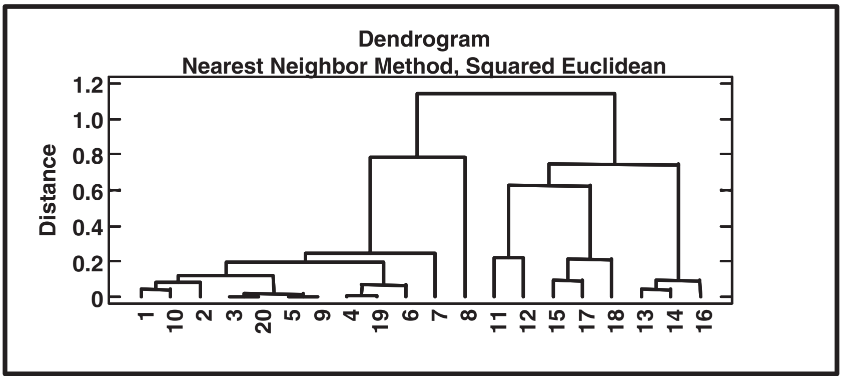

Figure 3. Dendrogram showing the results of a hierarchical cluster analysis according to Cu and Fe concentrations in seafood samples.

natural groupings among the samples looking for one preliminary way to study the data structure. Cluster analysis describes the similarity between seafood samples in terms of their copper and iron concentrations. In this case, a matrix consisting of the squared Euclidean distance was used as a similarity matrix. A hierarchical agglomerative method (Ward'f method) was used to obtain clusters by using the statistical software packaging Statgraphic v. 4.1. As described by Meloun *et al.* [19], this procedure takes into account in each step the heterogeneity of the deviation (the sum of the squares of the distance of an object from the varycentre of the cluster). The results obtained showed that Cu and Fe concentrations contain useful information for the category classification of the seafood samples. Seafoods can be classified into two main clusters (figure 3). The first consists of mussels and clams. The seafoods that contain the highest Cu and Fe concentrations form this cluster. The other cluster is found in samples, which show lower Cu and Fe concentrations. This cluster shows three different subclusters in decreasing order of Cu and Fe concentrations: one found in tuna and sardine, one found in prawn, scallop and cockle, and one found in hake, crab and razor-shell.

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