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FOOD ANALYSIS

Automated Method for the Total Creatinine Determination in Dehydrated Broths

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Abstract: In order to improve the quality control of dehydrated broth, a new automated method was developed to determine total creatinine in dehydrated broths. The sample pretreatment was coupled on-line with the Flow Injection Analysis (FIA) system for analyte determination by the classical Jaffé reaction, stopped flow methodology, and spectrophotometric detection. The time consumed was reduced from 7 h, which is necessary with the official method, to 25 min. The calibration graph is linear between 0.342–1.368 mg creatinine/100 mL. The relative standard deviation (RSD%) was 1.7%, the sample throughput was 7 h^{-1}, and the detection limit was 0.185 mg creatinine/100 mL. The validation of the proposed method was carried out with real samples. The obtained results were compared with those obtained from the Association of Official Analytical Chemists (AOAC) reference method.

Keywords: Creatinine, meat product, automated analysis, FIA

INTRODUCTION

Creatine, present in muscle tissue, is an α-methyl guanidine acetic acid. Creatine becomes creatinine when it loses one molecule of water. An assay is used to detect the presence of meat extract in a food product (Belitz and Grosch 1999).

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Argentina is one of the most important exporters of meat and meat products, so an adequate analytical methodology is necessary to determine total creatinine because the content of this analyte is an index of the meat's quality (Artuso et al. 1984).

The Argentina Food Code establishes the minimum value for this parameter in different kind of meat products such as soup, meat extract, and dehydrated broth (Código Alimentario Argentino, Article 440).

To carry out this determination, the creatine content in the samples must be converted into creatinine by acidic hydrolysis. The reference method to quantify total creatinine is based in the Jaffé reaction; and it includes the sample treatment, which is tedious and time consuming (AOAC 1990).

Chromatographic (Stokes and O'Connor 2003; Pobozy et al. 2003) and amperometric (Stefan et al. 2003) methods are available in the literature to determine total creatinine in biological fluids. Nevertheless, the spectrophotometric methods based in Jaffé reaction are the most commonly used (Artuso et al. 1984; Gennaro et al. 1995; Walsh and Dempsey 2002).

A few papers have reported matrixes such as soup or dehydrated broths and a few by using flow injection methodology (Gutiérrez et al. 1989; Del Campo et al. 1998; Rui et al. 1993; Campins-Falco et al. 2001). In these papers the sample was treated by the official method.

The aim of this work is to propose a novel method to determine creatinine. Treatment of the samples was carried out in a focused microwave oven (MWO) (Acebal et al. 2005); and the obtained solution was introduced on-line into the FIA system, where the spectrophotometric determination of creatinine was done by using a stopped-flow methodology. Therefore, the classical Jaffé determination was modified to be adapted to the continuous flow mode.

In this way, a new automated method that includes the continuous treatment of the samples and determination of total creatinine was developed to contribute to the control of this parameter in different meat products.

The proposed method was validated by comparing the obtained results with those generated by the reference batch method (AOAC 1990), when both were applied to real samples.

EXPERIMENTAL

Instrumentation

A focused microwave oven (MWO) digester (Prolabo Maxidigest MX 350) with a frequency of 2450 MHz and a maximum power of 300 W, equipped with a PTFE reactor (length 5 m, inner diameter 0.8 mm), was used for the samples' treatment. A Perkin-Elmer Lambda 2 spectrophotometer coupled to a FIAS 300 Perkin Elmer was used to measure the signal at 500 nm. A Hellma 178-010 QS flow cell (inner volume 18 μL) was also used. A Julabo U3 thermostat was used.

Reagents and Solutions

All solutions were prepared with ultra pure water (18 MΩ) and the chemicals were of analytical reagent grade from Merck.

A 7.56×10^{-3} M stock creatinine solution in 0.1 mol/L HCl was prepared and stored at 4°C. The spiked creatinine solution (1.7×10^{-4} mol/L) and working standard solutions were prepared daily by diluting this stock solution.

By weighting suitable amounts of NaOH, solutions of different concentrations were prepared: A Solution: 0.55 mol/L; B solution: 1 mol/L; and C solution: 0.3 mol/L.

A concentrated solution (41 mmol/L) of picric acid was prepared by dissolving 7.1139 g (Sigma) in 500 mL of water. The working solution was prepared daily by an adequate dilution of the concentrated solution.

The samples were purchased in different supermarkets in Argentina and they are labeled as 1, 2 and 3.

Samples Pretreatment

The pretreatment of samples was carried out by applying a new method, which was developed by the authors (Acebal et al. 2005). A suitable amount of the sample was dissolved in 1.5 mol/L HCl. It was stirred for 10 min at 70°C–80°C. Thus, the analyte was completely dissolved, and this solution was introduced into a focused microwave oven in continuous. Then, the sample flow was irradiated and the acidic hydrolysis took place. The solution in the outlet of the MWO was cooled before going into the FIA system for the spectrophotometric determination.

Procedure

Figure 1a shows the FIA system, which was designed to prepare the standard and sample solutions that will be used to load the loop of the second FIA assembly.

The sample that came out of the MWO was introduced into the FIA system by a peristaltic pump. This stream merged with a 0.55 mol/L NaOH solution (A solution) stream in the R1 reactor and a pH similar to the standard solutions was attained.

A spiked creatinine solution (1.5×10^{-4} mol/L) stream was flowing through the system in order to increase the signal sensitivity. A selection valve (SV) was used to let that standard solutions or samples solution merge alternatively with the spiked creatinine solution in the R2 reactor. This generated stream merged with 1 mol/L NaOH (B solution) in the R3 reactor; then this stream went to load the loop of the injection valve (IV) depicted in Fig. 1b.

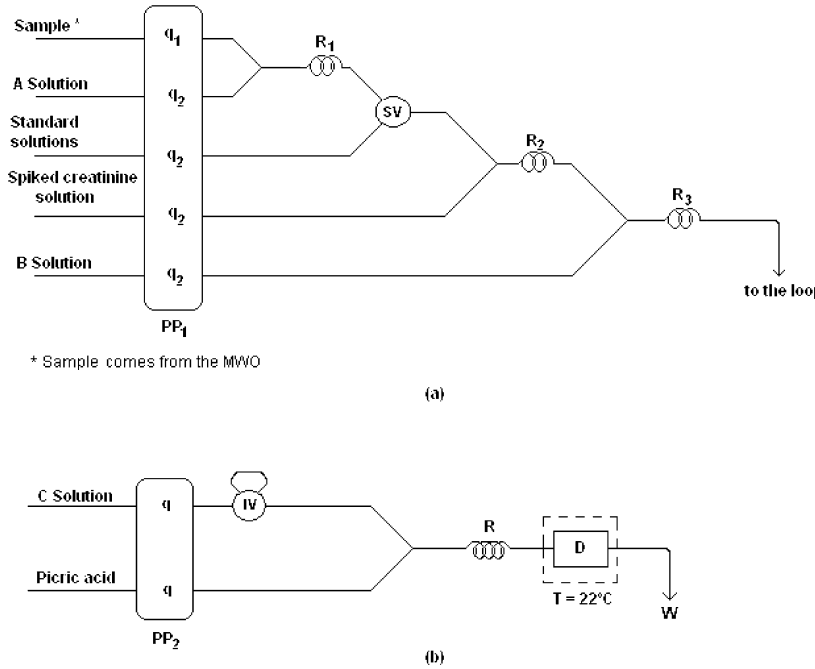

Figure 1. (a) Flow injection system to prepare the standard and sample solutions. PP1: peristaltic pump, SV: selection valves, q1 and q2: flow rates, R1–R3: reactors. (b) Flow assembly for total creatinine determination. PP2: peristaltic pump, IV: Injection valve, q: flow rate, R: reactor, D: spectrophotometer, W: waste.

In this FIA assembly (Fig. 1b), the standard/sample solution was injected into 0.3 mol/L NaOH carrier solution (C solution). This stream merged with the picric acid solution in the R reactor. The sample plug reached the flow cell that was thermostated at 22°C $\pm$ 0.1°C. The flow was stopped 2 s after the residence time (delay time 17 s). A stop time of 495 s allowed the kinetic curve to be monitored at the maximum absorption wavelength (500 nm). After that time the pump is turned on automatically and the baseline is restored.

The absorbance increment over the selected interval time was measured.

RESULTS AND DISCUSSION

Optimization of Chemical and FIA Variables

Chemical Variables

Different solutions of NaOH were used in the system and they were labeled as solutions A, B, and C in Fig. 1.

Solution A: The sample solution in the outlet of the MWO was acidic, but it was necessary to adjust to a pH between 5–7. So, different concentrations of solution A were tested. The studied range was 0.1 mol/L–0.6 mol/L and the optimum value was 0.55 mol/L.

Solution B: As the colorimetric reaction takes place in alkaline medium, we decided to introduce a stream of NaOH that merged with sample or standard solutions in the R3 reactor before loading the loop. One mol/L of this solution was the optimum value. At higher concentrations an unstable baseline was obtained, and at lower concentrations the suitable medium was not obtained.

Solution C: The FIA signal was improved when NaOH solution was used as a carrier instead of water. Therefore, the concentration of this solution was optimized between 0.1 and 1 mol/L, and the optimum value was 0.3 mol/L.

A creatinine solution was introduced in the system to raise the FIA signal. Using this an important increase in the sensitivity was attained. Two different concentrations of the spiked creatinine solution were tested (1.5×10^{-4} mol/L and 3.4×10^{-4} mol/L). There was no difference in the increment of the signal with both concentrations, so the lower one was selected.

The concentration of picric acid solution was tested from 1.7×10^{-3} to 1.2×10^{-2} mol/L, and the best signal was obtained with 8.3×10^{-3} mol/L.

FIA Variables

The FIA variables were optimized. The studied ranges and optimum values are shown in Table 1.

Table 1. Studied ranges and optimum values for FIA variables

Variable	Studied range	Optimum value
Sample flow rate (mL/min)	0.40–1.37	0.55
Solution A flow rate (mL/min)	1.30–3.92	1.92
Solution B flow rate (mL/min)	1.30–3.92	1.92
Standard solution flow rate (mL/min)	1.30–3.92	1.92
Spiked creatinine solution flow rate (mL/min)	1.30–3.92	1.92
Solution C flow rate (mL/min)	1.37–2.90	2.62
Picric acid flow rate (mL/min)	1.37–2.90	2.62
Reactor length (R1) (mm)	80–300	100
Reactor length (R2) (mm)	80–300	100
Reactor length (R3) (mm)	80–300	100
Reactor length (R) (mm)	100–300	150
Sample volume (μL)	50–400	300
Cell temperature (°C)	20–50	22
Delay time (s)	15–19	17
Stopped-flow time (s)	349–495	495

Analytical Parameters

The calibration graph was linear between 0.342–1.368 mg creatinine/100 mL and the obtained regression equation was $A = 0.039 \pm 0.002$ (mg total creatinine/100 mL) + 0.065 ± 0.002, $R^2 = 0.995$. The detection limit (LOD) was 0.185 mg creatinine/100 mL, calculated from the calibration graph (Miller and Miller 1993) and the quantification limit (LOQ) was 0.346 mg creatinine/100 mL calculated from 10 measurements of the blank and by using the IUPAC criterion (Currie 1995). The relative standard deviation (RSD%) was 1.7%, obtained from 14 replicates of real samples and the sample throughput was $7\,h^{-1}$.

Determination of Total Creatinine in Real Samples

To determine total creatinine in real samples, different brands of dehydrated broths were used. The pretreatment of samples was done in the MWO, as described in samples pretreatment. The total creatinine was determined by the proposed method and the same samples were analyzed by using the AOAC method.

The obtained results for the different samples, after applying both methods, are shown in Table 2. In order to validate the proposed method through real samples, a comparison between the results of both methods and different samples was carried out by using a two-way ANOVA test. The F value obtained in this way compared with the critical F ($F_{calculated} = 3.03$ and $F_{critic} = 4.17$) showed no significant statistical differences for a 95% confidence level. Thus, we concluded that the proposed method is traceable to the reference method.

Table 2, also shows the standard deviations obtained from five replicates in intermediate precision conditions. The proposed method has lower values

Table 2. Results obtained by using the proposed method and the reference method (AOAC)

		Creatinine (mg/dm^{-3}) ($\bar{X} \pm S$)[b]	
Sample	Type	Proposed method	AOAC method
Dehydrated broth 1[a]	Meat	29.13 ± 0.03	29.95 ± 1.31
	Chicken	9.67 ± 0.02	9.55 ± 0.06
	Stew	19.01 ± 0.01	18.32 ± 1.55
Dehydrated broth 2[a]	Meat	38.30 ± 0.04	36.50 ± 1.51
Dehydrated broth 3[a]	Meat	38.13 ± 0.50	37.35 ± 1.42

[a] 1, 2 and 3: different commercial brands.
[b] n = 5.

than those obtained with the AOAC method, this indicates that the reproducibility of our method is better. This improvement on the reproducibility is owing to an automated method where the pretreatment of samples is simpler and easier than that of the official method.

CONCLUSIONS

We proposed an automated method for total creatinine determination in dehydrated broths. It is fast and, as the sample pretreatment is coupled on-line to the FIA assembly, the automated degree of the analytical method is increased. Therefore, the sample throughput is higher.

The determination of total creatinine in dehydrated broth by using the AOAC method consumed 20 min, while only 8 min is necessary for our FIA method. By considering the whole method, that include the sample pretreatment, the AOAC method consume 7 h and the proposed method only 25 min.

Moreover, a better reproducibility than the official method was obtained and considering that a spectrophotometric technique is available in all kinds of laboratories, the proposed method may be considered as an alternative to the official method.

The validation of the process showed that the method could be adapted without risk in control laboratories.

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