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A new and fast continuous method for the pre-treatment of dehydrated broth for total creatinine determination

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Abstract

The quantification of total creatinine contributes to the quality control in meat products. The reference method for dehydrated broths treatment is tedious and takes approximately 6 h. A fast, simple and relatively safe procedure to the pre-treatment of these samples was developed by using a focussed microwave oven which works in continuous way. The optimization of the different variables for the pre-treatment was made and the validation of the method was carried out in order to ensure the accuracy of the obtained results.

Keywords: Dehydrated broth; Microwave oven; Creatinine; On line pre-treatment

1. Introduction

Creatine and creatinine are characteristic constituents of muscle tissue and their assays are used to detect the presence of meat in food product as soup and dehydrated broth (Belitz & Grosh, 1987).

The Argentine Food Code defines dehydrated broth as a product resulting from the combination of meat and its extracts, fat, edible salt, condiments, spices and flavour accent agents (Código Alimentario Argentino, Art. 440).

The creatinine content is appropriate to be used as a quality indicator for meat products (Dvorák, 1981). Usually, total creatinine and free creatinine are determined by the classical Folin method (Folin, 1914) which is based on the the chemical reaction between the analyte and the alkaline picrate (Jaffe, 1886).

To determine total creatinine is necessary an acidic hydrolysis step to turn creatine into creatinine. The oficial method of AOAC for the pre-treatment of these kinds of samples involves several previous operations (weighted, grinding, dissolving, evaporating, etc.) in order to obtain free creatinine (AOAC, 1990). Then, a prolonged heating in acidic medium is required to convert creatine into creatinine, and by this way the total creatinine in the sample can be determined. As can be seen, this pre-treatment is unpleasant, tedious and time consuming procedure. Moreover, the sample contamination may occur during this treatment and an improper handling may easily introduce errors (Burguera & Burguera, 1998).

In the last years, some extraction techniques have been developed to improve the precision of analyte recoveries and to reduce the extraction time and sample preparation cost. Such techniques include microwave assisted extraction (Ahmed, 2003) for the treatment of a lot of different kinds of samples including food (Caballo-López & Luque de Castro, 2003; Oliveira, Sartini, & Guidetti Zagatto, 2000).

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The aim of this work is to propose a new continuous flow method for the pre-treatment of dehydrated broth by using a focused microwave oven which is commanded by a computer.

The human participation was only to weight a suitable amount of sample. As soon as the sample was stirring during a determined time, the analyte solution was introduced into the MWO at a fixed flow rate and the hydrolysis took place during the irradiation of the sample. After this treatment, the solution was collected outside the MWO and creatinine was determined by the classical Jaffé reaction. The proposed method was validated by comparing measurements obtained from samples that were treated by using the official method and microwave assisted technique.

2. Experimental

2.1. Reagents and solutions

All the 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 creatinine stock solution in 0.1 M HCl was prepared and stored at 4 °C. Working standards solution were prepared daily by diluting this stock solution.

Commercial samples of different trademarks were used and they were labeled A, B and C. The A labeled samples were three different dehydrated broths and their origins were meat, chicken and stew. Besides, samples B and C were both from meat.

2.2. Instrumentation

The hydrolysis of creatine was carried out in 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). The microwave digester control was exercised by the FIAS software of Perkin–Elmer.

Samples were introduced into the MWO in by a Gilson Minipuls 3 peristaltic pump.

A heater-magnetic stirrer (Decalab) was used.

Spectrophotometric measurements were obtained at 500 nm, with a Perkin–Elmer Lambda 2 spectrophotometer using a 10 mm quartz cell.

2.3. Sample treatment

Sample treatment was carried out by dissolving 0.5 g of the sample, previously homogenized, in a baker with 25 ml of 1.5 M HCl solution. Then, it was stirred during 10 min at 70–80 °C to let the analyte dissolve completely. Under these conditions, the solution was introduced into the MWO at 2.32 ml min^{-1}. The sample stream was before filtered on-line by using an acetate column (length 3 cm, inner diameter 0.65 cm). Then, it was irradiated at 40% power (300 W). By this way, the acidic hydrolysis took place. The solution was then cooled by using a water cooler (i.d. 1 mm, length 2 m) in order to prevent the chemical reaction from going forward (Fig. 1).

An aliquot of 2.0 ml was taken, alkalinized with 2 M NaOH solution (pH 11–12) and diluted to 10 ml with water. Then, a suitable volume of this dilution was used to determine creatinine in accordance with the standard method (AOAC). The obtained calibration curve was $A = 0.925$ [mg total creatinine/100 ml] + 0.0011, with $R^2 = 0.999$.

The optimization of variables was carried out with a real sample which was used for that purpose. The total creatinine concentration of this sample was obtained by the AOAC method. This concentration was then used as a reference value to determine the optimum values for each variable.

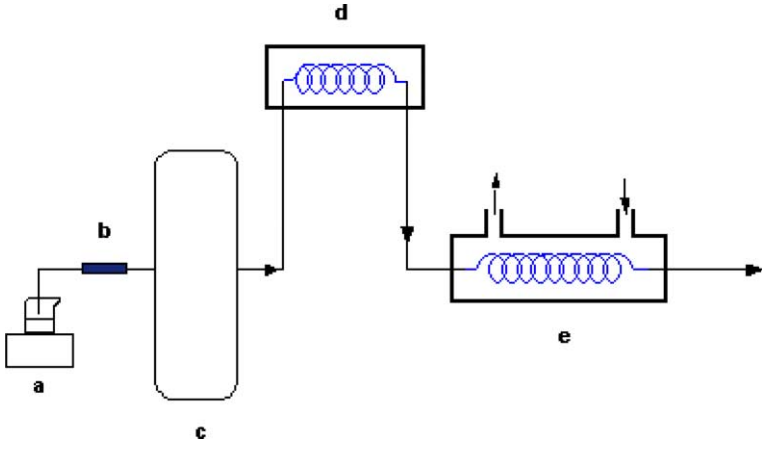

Fig. 1. Sample pre-treatment system: a: magnetic stirrer; b: acetate column; c: peristaltic pump; d: reactor; e: cooler.

3. Results and discussion

3.1. Optimization of sample treatment variables

The univariate method was used to carry out the optimization of the variables for the sample treatment. The optimization of each variable was done by triplicate.

3.1.1. Concentration of HCl

In order to determine total creatinine, an acidic hydrolysis was required to turn creatine into creatinine. The adequate HCl concentration was selected to prepare the sample solution that will be introduced in the MWO. This variable was tested from 0.5 to 2.5 M and the optimum value was 1.5 M.

3.1.2. Stirring time and temperature

As creatine and creatinine are both water soluble, the sample solution needs a suitable stirring time to become stable. Two different times (10 and 20 min) were tested. After 10 min of stirring, the sample solution showed an acceptable solubilization. Besides, a high temperature was favorable for the sample solubility. Different temperature ranges were proved and the best was around 70–80 °C. The control of this variable was not critical but it is important not to attain the boiling point.

3.1.3. Flow rate

Flow rates were studied in the range 0.78–2.32 ml min^{-1} with fixed microwave power of 35%. The lowest flow rate showed a higher concentration value but a high pressure was observed in the system. By increasing the flow rate, the obtained concentration values were slightly lower and these drawbacks produced for the high pressure disappeared. Thus, the highest flow rate was chosen as a compromise between the maximum concentration and the sample throughput.

3.1.4. Microwave power

Working with the optimum values obtained for the above studied variables, the microwave power was studied between 25% and the maximum value, 40%. The best results were obtained with 40%.

3.1.5. Filtration step

A column (a tygon tube of 3 cm length and 0.65 cm inner diameter) packed with cellulose acetate was used to remove some stuff as vegetables, fat, etc., that sometimes are found in dehydrated broths. The filtration was carried out on-line. Two different positions for the column were tested in the system. Before the stream of the sample went into the MWO and after the sample stream had passed thought the MWO and the cooler.

The obtained results showed a better reproducibility when the sample filtration was carried out before introducing the sample in MWO. On the other hand, when the filtration was done after the MWO treatment some drawbacks with the normal flow in the MWO reactor were obtained.

Table 1
Comparison of the obtained result when the treatment was carried out by the proposed method (MWO) and the reference method (AOAC)

Sample	Type	Creatinine (mg dm^{-3})		Difference between means
		MWO ($\bar{x} \pm s$)[a]	AOAC ($\bar{x} \pm s$)	
Dehydrated broth A	Meat	29.95 ± 1.31	27.47 ± 1.10	−2.48
	Chicken	9.34 ± 0.47	10.03 ± 0.86	0.69
	Stew	18.62 ± 1.82	18.47 ± 0.86	−0.15
Dehydrated broth B	Meat	37.35 ± 1.42	38.90 ± 2.80	1.55
Dehydrated broth C	Meat	36.04 ± 1.66	36.95 ± 3.45	0.91
				$\bar{d} = 0.10$
				$s = 1.57$

A–C, different commercial brands.
[a] $n = 5$.

3.2. Validation of the sample treatment

To carry out the validation of the proposed method, five different commercial dehydrated broths were used. The obtained results for total creatinine by the MWO hydrolysis are compared with those obtained by the reference method (Table 1). As the samples contain different amounts of creatinine, a comparison of the means of the differences between the paired observations was performed (Massart et al., 1997).

The t test of paired samples was applied on the data shown in Table 1. The $t_{calculated}$ value was 0.148 and the t_{critic} value was 2.78 at 95% confidence level and four degrees of freedom. It can be concluded that at this confidence level there were no significant differences between the two methods for the treatment of the samples.

4. Conclusions

The proposed continuous method is fast, simple and efficient for the treatment of the dehydrated broths for the determination of total creatinine. The main advantage is that it takes less than 15 min per sample, while the reference method takes 6 h. Besides, this proposed method shows an important lower consumption of sample and HCl solutions.

Moreover, this automated procedure may be useful for coupling on line to continuous flow determination methods.

The fact of obtaining creatinine content from different samples by applying the AOAC standard method to compare with those obtained with the proposed method implies a good way for proving traceability.

The obtained results show that systematic errors were not present. Thus, the method is traceable to the reference method.

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