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A new procedure for bovine milk digestion in a focused microwave oven: gradual sample addition to pre-heated acid

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Abstract

Milk samples can be efficiently digested using a focused microwave oven, however the conventional procedure of addition of concentrated acids to the liquid sample leads to digestates with elevated acidity and residual carbon concentrations. In this work a focused microwave oven was applied for acid digestion of bovine milk samples using a conventional and an alternative procedure based on gradual sample addition to hot and concentrated acids. A two-level 2^3 full factorial design experiment with eight runs was carried out to evaluate the optimum experimental conditions for reducing both the residual carbon and the final acidity of digestates. The three studied parameters were: temperature of the digestion medium for sample addition, addition of sulfuric acid before the sample or during the first step, and number of aliquots of the sample gradually added. The best conditions were attained by adding small aliquots of milk (ten-fold a volume of 0.5 ml added during 5.0 min) to a digestion mixture containing 3.0 ml nitric acid plus 1.0 ml sulfuric acid heated at 105 °C. It was demonstrated that the digestion efficiency of the alternative procedure was better than the conventional procedure, i.e. 98 and 80%, respectively. The alternative procedure was applied for determination of Ba, Ca, Cu, K, Mg, Na, P, and Zn in whole and non-fat bovine milk. The accuracy was proved using two certified reference materials (whole and non-fat milk powder).

Keywords: Focused microwave-assisted digestion; Sample addition; Pre-heated acid; Bovine milk

1. Introduction

One of the main routes of evolution in sample preparation is based on the development of microwave-assisted procedures. Microwave-assisted digestion is a well-accepted strategy for converting samples in representative solutions suitable for instrumental analysis. Microwave energy has been successfully used for conventional total digestions and for partial digestions or extractions before speciation analysis [1,2].

Microwave-assisted procedures can be implemented using either cavity- or focused ovens. There are procedures established employing each one of these ovens and some of them are officially recommended by governmental agencies [3,4]. However, there is a recent trend towards the development of new procedures based on less conventional approaches, such as microwave combustion [5,6], combination of ultraviolet and microwave radiations [7], simultaneous digestion of large number of samples in low-pressure reaction vessels [8], vapour-phase digestion [9–13], and use of diluted acids [14]. These procedures were implemented either using cavity-ovens [5–10] or focused ovens [11–14]. These developments deal with different limitations of conventional microwave-assisted procedures, such as treatment of solid

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samples [5,6], low energy of microwave radiation [7], sample throughput [8], analytical blanks [9–14], and digestates with low content of acids [14].

The main limitations of the focused microwave ovens are the great amount of acids needed and the requirement of sulfuric acid (boiling point 330 °C, [15]) for reaching elevated temperatures due to the conventional operation of the commercial ovens at normal pressure, despite a proposal of a prototype able to operate at high pressures [16].

Using focused microwave ovens, it is possible to program the addition of reagents at any time during the digestion allowing sequential acid attacks, handling of large samples that can generate huge amounts of gases mainly when working with organic materials, and safety due to operation at atmospheric pressure [17]. Additionally another attractive feature of the focused microwave oven is the high-efficiency transference and precise control of the energy delivered to the sample.

The procedure recently proposed is based on gradual addition of the sample to microwave-heated concentrated acids [17,18]. Using this strategy, it was demonstrated that it is possible to obtain an efficient digestion for large volumes of samples even when employing small volumes of concentrated acids. This procedure can be implemented using standard commercial equipment. In a review [17], it was emphasized that the proposed procedure presents the following characteristics:

- each sample aliquot is at least partially digested before adding the next one;
- digestion is carried out in a more concentrated acid medium because the reagent is less diluted by the sample solvent. It means that the acid is in excess compared to the sample during all digestion steps;
- hot concentrated acids can generate reactive radicals that can accelerate the digestion process;
- the heat generated by exothermic reactions promotes a fast heating of the reaction medium;
- lower blank values and better detectability owing to the lower dilution of digestates.

In this work, a focused microwave oven was applied for acid digestion of bovine milk samples using a conventional and an alternative procedure. The effects of the sequential addition of sample aliquots in microwave-heated concentrated acids, such as nitric and sulfuric acids, and temperature–time programs were examined taking into account the residual carbon content (RCC), elemental recoveries, and final acid concentration of the digestates. A two-level 2^3 full factorial design experiment with eight runs was carried out to evaluate the main variables and the interactions between them. The three studied parameters were: temperature of the acid digestion medium for sample addition; addition of sulfuric acid before the sample or during the first step; and number of aliquots of the sample gradually added. The resulting digestates were suitable for introduction in an axially viewed inductively coupled plasma optical emission spectrometer (ICP OES) using a V-groove nebulizer. Residual carbon was also determined by ICP OES [19,20].

Table 1
Instrumental parameters for ICP OES analysis of bovine milk digestates

RF forward power (kW)	1.3
Argon gas flow rates (l min^{-1})	
Plasma gas	15
Auxiliary gas	1.5
Nebulizer gas	0.7
Integration time (s)	1.0
Stabilization time (s)	15
Reading time (s)	1
Replicates	3
Nebulizer	V-groove
Spray chamber	Sturman-Masters
Spectral lines (nm)	Ba II 455.407, Na I 589.586
	C I 193.024, P I 177.432
	Ca II 317.936, Zn I 213.858
	Cu I 324.758
	K I 766.498
	Mg I 285.209

2. Experimental

2.1. Apparatus

Axially viewed simultaneous ICP OES (Vista AX, Varian, Mulgrave, Australia) equipped with a charge coupled detector was used to determine all selected elements. The spectrometer provided wavelength coverage from 167 to 785 nm with an optical system purged with argon and a polychromator with Echelle grating thermostated at 34 °C. The cool plasma tail was removed from the optical path using an end-on gas to purge the plasma–spectrometer interface. All instrument characteristics were discussed in a previous paper [21]. The ICP OES operating conditions are given in Table 1.

The acid digestions were performed using an open focused microwave oven STAR 6 (Simultaneous Temperature Accelerated Reactions, CEM, Matthews, NC, USA) with borosilicate glass vessels. Reaction vessels were cleaned with nitric acid (10% v/v) and rinsed with deionized water.

An element analyser (Fisons EA 1108 CHNS-O, Italy) was used for determining the original carbon contents in milk samples. A sub-boiling apparatus (subPUR, Milestone) was used to purify concentrated nitric acid.

Table 2
Conventional heating program for whole milk sample digestion in a focused microwave oven Initial addition: 10.0 ml HNO_3 + 3.0 ml H_2SO_4 for 2.5 ml of milk[a]

Step	T (°C)	t_{ramp} (min)	$t_{plateau}$ (min)	Reagent V (ml\type)	Aliquot (ml)
1	110	3	0	–	–
2	180	5	5	–	–
3	180	0	10	10 /H_2O_2	0.5

[a] Final dilution: 50.0 ml.

Table 3
Heating program for whole milk samples in a focused microwave oven using gradual sample addition to pre-heated acid Initial addition[a]: 3.0 ml HNO_3 or 3.0 ml HNO_3 + 1.0 ml H_2SO_4

Step	T (°C)	t_{ramp} (min)	$t_{plateau}$ (min)	V (ml)/sample or reagent type	Aliquot (ml)
1	70 or 105[a]	5	5	5 /milk	0.5 or 2.5[a]
2	105	0	1	1 /H_2SO_4[a]	1.0
3	170	2	1	–	–
4	170	0	10	10 /H_2O_2	1.0

[a] Parameters evaluated by factorial design.

Table 4
Factors and levels used in the factorial design experiment

Factors	High level (+)	Low level (–)
Temperature (°C)	105	70
H_2SO_4	Before sample	Step 2
Number of aliquots	10	2

2.2. Reagents and solutions

All solutions were prepared using analytical grade reagents and Milli-Q® distilled and deionized water (Millipore, Bedford, MA, USA). Nitric acid (Carlo Erba, Italy), sulfuric acid, and hydrogen peroxide (both Mallinckrodt, Mexico City, Mexico) were used for performing the microwave-assisted digestions.

A carbon stock solution containing 5.0% m/v C in aqueous medium was prepared using urea (CH_4N_2O, Reagen, São Paulo, Brazil) for RCC determination. The analytical curve (0.05, 0.10, and 0.25% m/v C) was prepared in 1.4 mol l^{-1} HNO_3 by proper dilution of the stock. Yttrium was added to all reference solutions and samples as internal standard in a final concentration of 1.0 mg l^{-1}. Details of this procedure were already presented [20].

Reference solutions for the analytical curve were prepared by suitable dilution of 1000 mg l^{-1} monoelement stock solutions (Spex CertiPrep, Metuchen, NJ, USA) containing Ba, Ca, Cu, K, Mg, Na, P, and Zn in 1.4 mol l^{-1} HNO_3, similar to the final acid concentration of the digestates.

2.3. Reference materials and samples

Standard reference materials from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), whole milk powder SRM 8435 and non-fat milk powder SRM 1549, and whole and non-fat bovine milk samples purchased in a local market were digested using the proposed procedure.

2.4. Sample preparation

2.4.1. Conventional procedure

The conventional heating program used for digesting milk samples in a focused microwave oven is described in Table 2. A volume of 2.5 ml of milk was introduced into a borosilicate vessel, then 10.0 ml of concentrated HNO_3 and 3.0 ml of concentrated H_2SO_4 were mechanically added by action of the equipment syringe pump. After digesting, solutions were transferred to volumetric flasks and diluted to 50.0 ml with water.

2.4.2. Alternative procedure: gradual sample addition to pre-heated acid

The heating program developed is shown in Table 3. The heating program was established by investigating the following variables: temperature for sample addition, addition of sulfuric acid, and number of sample aliquots (Table 4). The design matrix is shown in Table 5. The effect caused by higher temperatures during the sample introduction step was not evaluated to avoid pronounced losses of the digesting mixture by evaporation. The addition of sulfuric acid to the hot digestion mixture was tested before introducing the sample during the step 2. The total volume of the whole milk sample (5.0 ml) was added either in ten successive aliquots of 0.5 ml or in two successive aliquots of 2.5 ml. It is expected that a higher number of aliquots gradually added to the digestion mixture should lead to a better efficiency of digestion due to the reagent/sample ratio. The sequence of experiments carried out is shown in Table 5.

Table 5
Design matrix and results for residual carbon and acidity of the digestates (final volume: 15.0 ml)

Experiment	Initial temperature (°C)	Addition of H_2SO_4	Number of sample aliquots	Residual carbon (%)	Acidity (mol l^{-1})
1	+	+	+	1.50	1.28
2	+	+	–	1.87	0.922
3	+	–	–	3.88	1.59
4	+	–	+	6.86	1.33
5	–	+	–	5.97	1.54
6	–	–	+	9.21	1.49
7	–	+	–	10.9	1.23
8	–	–	–	9.00	0.768

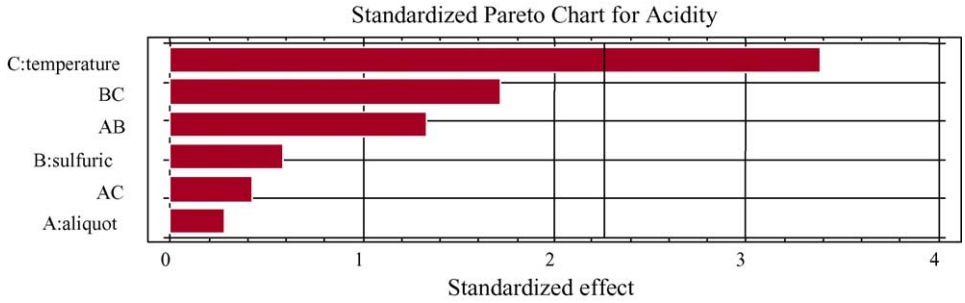

Fig. 1. Pareto's chart for acidity of the digestate.

In all experiments a total volume of 10.0 ml of H_2O_2 was added in 1.0 ml min^{-1} aliquots during the last step (step 4).

After digestions, solutions were transferred to graduated polypropylene flasks (Nalgene, Corning, NY, USA) and diluted to 15.0 ml with water. The final acid concentration of the digestates was determined by acid–base titration using a sodium hydroxide standard solution (0.9859 mol l^{-1}).

3. Results and discussion

The original carbon content of the whole milk sample was 6.7% and it was determined by elemental analysis. Further carbon measurements in the digestates were determined by ICP OES simultaneously to the analytes. Unless, when mentioned, all experiments were carried out using the whole bovine milk sample.

The experimental parameters of the factorial design experiment applied to the alternative procedure should be set to reach values as low as feasible for residual carbon and acidity. The factorial design experiment led to the results showed in Table 5 and the Pareto's charts are shown in Figs. 1 and 2 for acidity of the digestate and residual carbon content, respectively.

The variance test (ANOVA) for a significance level above 95% showed that only the temperature exerted a pronounced effect on the acidity of the digestate (Fig. 1). On the other hand, the number of sample aliquots, the step for addition of sulfuric acid, and the interactions between the temperature and the addition of sulfuric acid, the temperature and the number of sample aliquots, and the number of sample aliquots and the addition of sulfuric acid exerted a significant effect on the RCC (Fig. 2). The temperature as an isolated factor did not exert a significant effect and this can be explained considering that only the effect of the temperature in the first step during the sample addition was considered. It means that this variable per se is not critical because at the experimental conditions tested for gradual sample addition only a partial sample digestion at this step occurred as it will be shown later.

According to these results the best conditions for digestions employing the alternative procedure are:

- additions of a higher number of milk sample aliquots, i.e. 10-fold a volume of 0.5 ml is better than twice a volume 2.5 ml;
- sulfuric acid mixed with nitric acid should be added before the sample addition. It could be mentioned that the use of sulfuric acid alone was less efficient because the acidity of the digestates was greater in this condition;
- the temperature for sample addition should be set in 105 °C.

A surface response for RCC considering the number of sample aliquots and the step for addition of sulfuric acid is shown in Fig. 3. This surface response confirms that the best approach involves a higher number of sample aliquots and the addition of sulfuric acid before the sample.

Addition of successive aliquots of hydrogen peroxide at the last step increases the oxidation potential of sulfuric acid to 1.81 V by forming monopersulfuric acid, H_2SO_5 (Caro's

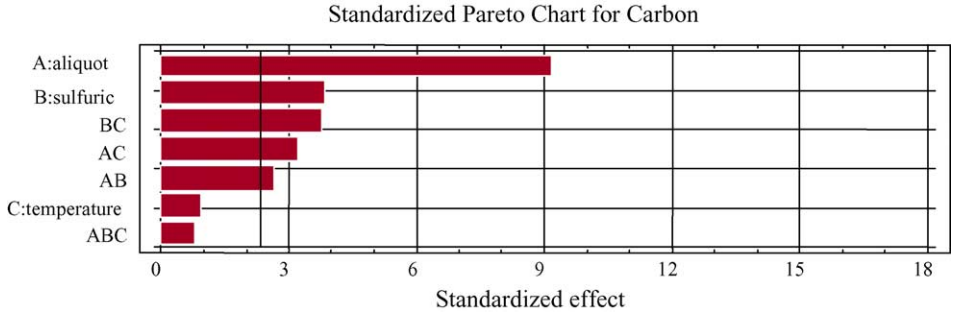

Fig. 2. Pareto's chart for residual carbon of the digestate.

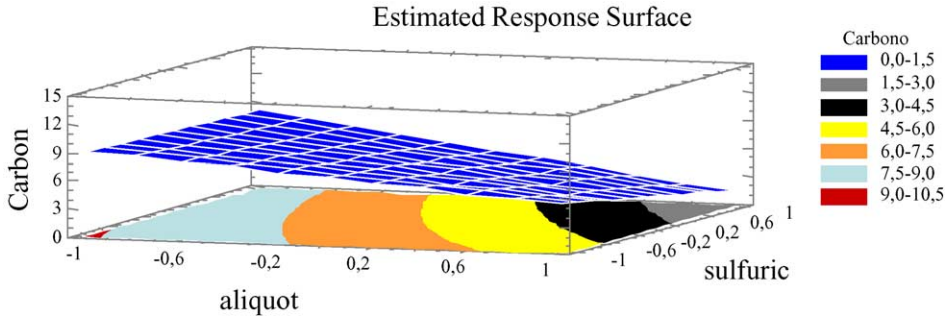

Fig. 3. Surface response for residual carbon content.

acid) [19]. A reduced volume of H_2SO_4 (i.e. 1.0 ml) was sufficient to decompose organic compounds. The difficulty to remove the excess of sulfuric acid after completion of the oxidation, due to its high boiling point, was minimized by using just a 1.0 ml volume, which was mostly consumed during the digestion. Generally, the charred residue produced by sulfuric acid treatment is further treated with another oxidant, such as nitric acid or hydrogen peroxide, which continues the decomposition by further oxidizing the partially degraded fragments [22].

It can also be mentioned that the temperature during the first step can increase up to 160 °C during short time intervals due to the occurrence of highly exothermic reactions when the sample is added. After this sudden and abrupt increment, the temperature fastly decreased to the set level and the temperature control was again assumed by the combined action of the infra-red sensor and the magnetron of the focused oven.

After establishing the optimum experimental conditions, the efficiency of the alternative procedure was compared with a conventional procedure. It is not totally correct to compare directly each heating program because times at each temperature and the volumes of the digestion mixture are completely different. So, an experiment was carried out employing only the first step in each condition, using a volume of 3.0 ml nitric acid and adopting a temperature of 100 °C. The temperature was kept during 6 min. In both cases, a volume of 5.0 ml whole bovine milk was digested. In the case of the alternative procedure, this volume was added in aliquots of 0.5 ml during the first 3 min of the heating program. This experiment also allowed to evaluate if there is at least a partial digestion at low temperature. The residual carbon contents were 81 and 66% for the conventional and alternative procedures, respectively. These results demonstrated that the performance has really improved when the sample is gradually added to the hot acid and even at a temperature of 100 °C, the process of digestion of easily oxidizable compounds starts. The efficiency of the partial digestion is 1.8-fold higher, when the sample was gradually added.

The final acidity of the digestate obtained using the conventional program (Table 2) was 2.4-fold higher than that obtained using the alternative program (Table 3). Comparing the focused oven conventional and the alternative procedures, the nitric and sulfuric acid volumes were reduced from 10.0 to 3.0 and from 3.0 to 1.0 ml, respectively. The highest dilution required by the conventional procedure for proper introduction of the digestate in the ICP OES using a pneumatic nebulizer affects negatively the detection power. In addition to the improvement of the detection power due to a lower dilution factor, the pre-heated acid procedure also reduced the generation of acid vapours during sample digestion.

Table 6
Residual carbon content (% RCC) of bovine milk samples digested in a focused microwave oven (mean ± S.D., $n = 3$)[a]

Milk sample	Conventional procedure	Alternative procedure
Whole milk	19.6 ± 0.3	0.979 ± 0.05
Non-fat milk	17.9 ± 1.7	1.72 ± 0.16

[a] Gradual addition of sample to pre-heated acid.

The RCC's determined using both procedures (Tables 2 and 3) are shown in Table 6. Using the conventional procedure, a digestion efficiency around 80% for non-fat and whole milk samples was observed. For non-fat and whole milk samples digested using the alternative procedure, the digestion efficiency was around 98% for both samples.

Elements determined by axially viewed ICP OES in two commercial samples digested using the alternative procedure proposed are shown in Table 7. The accuracy of the alternative procedure was evaluated using the standard reference materials NIST SRM 8435 Whole Milk Powder and NIST SRM 1549 Non-Fat Milk Powder (Table 8). According to a paired

Table 7
Analysis of digested bovine milk samples employing the procedure of sample addition to pre-heated acid (mean ± S.D., $n = 3$)

Element	Commercial milk samples (UHT)	
	Non-fat	Whole
Ba (mg kg^{-1})	0.110 ± 0.007	0.112 ± 0.009
Ca (g l^{-1})	0.911 ± 0.127	0.905 ± 0.056
Cu (μg l^{-1})	33.4 ± 2.9	34.3 ± 4.1
K (g l^{-1})	1.50 ± 0.06	1.44 ± 0.10
Mg (mg kg^{-1})	81.0 ± 4.7	74.6 ± 6.5
Na (mg kg^{-1})	391 ± 19	348 ± 28
P (g l^{-1})	726 ± 40	697 ± 42
Zn (mg kg^{-1})	2.84 ± 0.16	2.72 ± 0.24

Table 8
Analysis of milk standard reference materials using the procedure of gradual addition of sample to pre-heated acid (mean ± S.D., $n = 3$)

Element	NIST SRM 8435 Whole milk powder		NIST SRM 1549 Non-fat milk powder	
	Determined[a]	Certified	Determined[a]	Certified
Ba ($mg\,kg^{-1}$)	0.472 ± 0.030	0.580 ± 0.230	0.513 ± 0.017	–
Cu ($mg\,kg^{-1}$)	0.420 ± 0.115	0.460 ± 0.08	0.406 ± 0.142	0.7 ± 0.1
Ca (%)	0.907 ± 0.001	0.922 ± 0.049	0.980 ± 0.001	1.30 ± 0.05
K (%)	1.61 ± 0.01	1.363 ± 0.047	1.69 ± 0.01	1.69 ± 0.03
Mg ($mg\,kg^{-1}$)	791 ± 2	814 ± 76	1110 ± 10	1200 ± 30
Na (%)	0.415 ± 0.001	0.356 ± 0.040	0.464 ± 0.003	0.497 ± 0.010
P (%)	0.834 ± 0.002	0.780 ± 0.049	1.02 ± 0.01	1.06 ± 0.02
Zn ($mg\,kg^{-1}$)	25.9 ± 0.4	28.0 ± 3.1	39.3 ± 1.1	46.1 ± 2.2

[a] Milk powder: 0.2 g/12.5 ml H_2O.

t-test, most determined and certified values are in agreement at a 95% confidence level.

4. Conclusion

The digestion of milk samples was carried out efficiently in a focused microwave oven using either a conventional procedure or an alternative procedure based on gradual sample addition to pre-heated nitric and sulfuric acids. The latter led to a better efficiency of digestion for higher amounts of samples even when using lower volume of concentrated acids.

The possibility to program the gradual addition of sample aliquots to the microwave-heated-acid generated a drastic reaction condition compared to the conventional procedure. The advantages of the alternative procedure were lower dilution of the digested solution before ICP OES measurements and more complete digestion of higher volumes of samples using lower volumes of concentrated acids. This strategy expands the application of focused microwave oven and produces digestates more suitable to measurements using instrumental techniques, such as ICP OES.

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