Effect of acid concentration on closed-vessel microwave-assisted digestion of plant materials

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Abstract

The efficiency of microwave-assisted acid digestion of plants using different concentrations of nitric acid (2.0, 3.0, 5.0, 7.0 and 14 mol l⁻¹) with hydrogen peroxide (30% v/v) was evaluated by measuring the residual carbon content (RCC) using inductively coupled plasma optical emission spectrometry (ICP-OES) with axial viewing. Certified reference materials were used for evaluating the accuracy attained when 2 mol l⁻¹ HNO₃ was employed for digestion. Under all experimental conditions RCC values were always lower than 13% w/v, and even the highest concentration did not cause any interference with element recovery. It seems that the high pressure reached for closed-vessel operation improved the oxidative action of nitric acid due to consequent temperature increase, even when this reagent was not used at high concentrations. According to acid–base titration data, residual acid in the digestates varied from 1.2 to 4.0 mol l⁻¹, depending on the acid concentration initially added. It can be concluded that for plant materials, microwave-assisted acid digestion can be carried out under mild conditions, which implies that digestates do not need extensive dilution before introduction by pneumatic nebulization to ICP-OES. An additional advantage is the lower amount of residue generated when working with less concentrated acid solutions.

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1. Introduction

Closed-vessel microwave-assisted acid digestion has been extensively used for sample digestion. There are a huge number of publications dealing with this topic, and in most of them the experimental conditions adopted seem essentially empirical. Various parameters are relevant when a closed-vessel microwave-assisted digestion is promoted: sample mass, acid type, volume of reagents, oxidant mixture concentrations, temperature, pressure and time of heating. Several of these parameters have been studied to obtain a better understanding of the digestion process. Despite using different acids for sample digestion, HNO₃ is frequently employed due to its simple manipulation, facility of purification and oxidative capacity for organic compounds in biological samples [1–4].

Some authors recommended the use of 0.4–2.0 ml of HNO₃ for digesting 200 mg of sample [5–7], and others showed that a volume of 2.0 ml of HNO₃ is sufficient to perform an efficient digestion of 100 mg of sample by controlling only the temperature [1,8]. However, diluted HNO₃ solutions have been successfully used for microwave-assisted plant digestion, generating low blank values and low relative standard deviations [9–11]. Procedures using a low volume of acids or dilute acid solutions generally require H₂O₂ as an auxiliary oxidant agent. Hydrogen peroxide addition has been investigated, and a decrease in both solid residue and residual carbon content (RCC) was observed for sample digestates obtained using a higher volume of this reagent [12]. In addition, the use of higher amounts of H₂O₂ caused a build up of pressure, leading to efficient organic-matter oxidation at lower temperatures. However, for volumes higher than 5.0 ml, the solid residue and RCC remained constant, showing that high volumes of H₂O₂ are not imperative for efficient sample digestion [12,13]. Some procedures recommended a mixture containing 4:1 v/v HNO₃/H₂O₂ (both as concentrated reagents); others showed that a mixture composed of 2.0 ml of concentrated HNO₃ combined with 1.0 ml of H₂O₂ is efficient in digesting up to 300 mg of biological samples [14–18]. The possibility of decreasing volumes of reagents is attractive from the point of view of minimizing residues, cost reduction, decrement of blank values, and generating digestate solutions better suited to introduction by nebulization.

The feasibility of using dilute acid solutions and a reduced volume of hydrogen peroxide originates from both the high pressure and high temperature reached in closed vessels. Higher temperatures cause more complete destruction of the sample matrix and improved accuracy [19].

In the work presented here, samples of different species of *Paspalum*, a forage used for animal feeding, were microwave-assisted acid-digested in closed vessels using different combinations of HNO₃ and H₂O₂. The total volume of reagents was kept constant, but the concentration of HNO₃ was varied. The goal was to establish the minimum amount of reagents that could be used to attain efficient digestion. This parameter was correlated to residual carbon content, residual acidity of digestates, nuclear magnetic resonance (NMR) residual spectra, and element determination by inductively coupled plasma optical emission spectrometry (ICP-OES) with axial viewing. The effects of microwave heating-program parameters on the digestion efficiency were also evaluated.

2. Experimental

2.1. Instrumentation

A 6750 freezer mill (Spex Certiprep, USA) and a cutting mill (TE 048, Marconi, Brazil) fitted with a 20-mesh screen at the bottom of the cutting chamber were used for sample grinding.

A closed-vessel microwave digestion system (ETHOS-1600, Milestone, Italy) equipped with fiber optic temperature and pressure sensors was used for sample digestion.

Total carbon was determined in an elemental analyzer CHNS-O (EA 1108, Fisons Instruments, Italy) and an ICP-OES with axial view configuration (VISTA AX ICP-OES, Varian, Australia) was used for elemental analysis.

A digital potentiometer (Mettler Toledo, model 355, England) equipped with a combined Ag/AgCl, saturated KCl reference electrode and a
glass indicator electrode was used to perform acid–base titration. All measurements were carried out in triplicate.

The $^1$H and $^{13}$C NMR analyses were carried out using Bruker DRX-400 (9.4 T, Germany) and Bruker ARX-200 (4.7 T, Germany) equipment, respectively.

2.2. Reagents, standards and samples

All solutions were prepared using distilled and deionised water (18 MΩ cm, Millipore, USA). Sub-boiled distilled nitric acid (Milestone, Italy) and hydrogen peroxide 30% v/v (Mallinckrodt, Germany) were used for sample digestion.

The multielement reference solutions were prepared daily from 1000 mg l$^{-1}$ stock solutions (Titrisol®, Merck, Germany). All solutions were prepared on a laminar flow bench. Glassware and plasticware were soaked overnight in 10% v/v HNO$_3$, and rinsed three times with pure water before use.

Two standard reference materials, spinach leaves (NIST 1570a, National Institute of Standards and Technology, USA) and corn bran (NIST 8433), were employed to evaluate accuracy. Samples of Paspalum notatum were used to optimize the microwave program. After the program was established, it was applied to samples of citrus and mango leaves, and to three other species of Paspalum (P. regnellii, P. guenoarum, P. glaucescens).

The residual acidity of the digestates was determined by an acid–base titration using a sodium hydroxide solution standardized with potassium hydrogen phthalate. All titrations were carried out in triplicate.

2.3. Sample grinding, digestion and elemental analysis

Samples were first ground in a cutting mill, with further grinding in a freezer mill to reduce the particle size. The freezer mill program used a 2-min initial step for freezing and a grinding step of 2 min, followed by a cooling step of 1 min. The grinding and cooling steps were repeated three times.

Sample masses of 250 mg of Paspalum were microwave-assisted digested using 2 ml of HNO$_3$ solutions (2.0, 3.0, 5.0, 7.0 and 14 mol l$^{-1}$) and 1 ml of H$_2$O$_2$ (30% v/v) in perfluoroalcoxy (PFA) closed vessels. In another experiment using 2 ml of 14 mol l$^{-1}$ HNO$_3$, 1 ml of water was used instead of hydrogen peroxide.

The heating program was performed in five steps: (1) 2 min at 250 W; (2) 2 min at 0 W; (3) 4 min at 550 W; (4) 4 min at 650 W; and (5) 5 min at 750 W. Temperature and pressure sensors were used in all digestions. After digestion, samples and blank solutions were transferred to 10.0-ml volumetric flasks and the volume was made up with water.

Analytes and RCC were determined by ICP-OES with axial view configuration. The operating conditions are summarized in Table 1. Residual carbon was determined according to conditions previously established [19]. For comparison, total carbon was previously determined by elemental analyzer.
2.4. Analysis of residues by NMR

Samples of *P. notatum* were digested using different HNO$_3$ acid concentrations. The organic substances remaining in a volume of 2 ml of digestate were extracted using a mixture containing 2.5 ml of ethyl ether and 2.5 ml of chloroform. The organic phase was evaporated in a forced-air oven at 40 °C. Solid residues were recovered in 0.6 ml of CD$_3$OD and tetramethylsilane (TMS) was added as an internal standard. All $^1$H NMR acquisition spectra were obtained using the same conditions: spectral width, 5910 Hz; 10.5 μs for 90° pulse; acquisition time, 5.5 s; relaxation delay, 2.0 s; and 64 scans. The temperature was maintained at 25 °C. For comparison purposes, 0.3 μl of pentafluorobenzaldehyde was used as an internal reference and the integral values were automatically achieved. All $^1$H NMR spectra were processed using exponential multiplication (LB = 1.0 Hz) and zero filling. The $^{13}$C NMR spectra were acquired using the PENDANT [20] pulse sequence, with spectral width of 13513 Hz, acquisition time of 1.5 s, relaxation delay of 1.5 s and 25 000 scans.

### Table 2
Residual carbon amount in *Paspalum* samples

<table>
<thead>
<tr>
<th>Acid concentration (mol l$^{-1}$)</th>
<th>Residual carbon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>6.9 ± 2.2</td>
</tr>
<tr>
<td>7</td>
<td>10.3 ± 1.9</td>
</tr>
<tr>
<td>5</td>
<td>10.7 ± 2.0</td>
</tr>
<tr>
<td>4</td>
<td>10.5 ± 2.0</td>
</tr>
<tr>
<td>3</td>
<td>11.3 ± 2.0</td>
</tr>
<tr>
<td>2</td>
<td>11.3 ± 1.9</td>
</tr>
</tbody>
</table>

Samples were digested with 2 ml of different HNO$_3$ concentrations plus 1 ml of H$_2$O$_2$, media between all samples digested with each different acid solution, $n$ = 3.

### 3. Results and discussion

3.1. Effect of experimental parameters on digestion efficiency

The manual for the ETHOS 1600 microwave oven recommends the addition of 10 ml of concentrated HNO$_3$ and 1 or 2 ml of concentrated H$_2$O$_2$ for the digestion of 250 mg of plant sample [21]. However, previous studies showed that a solution with 2 ml of 14 mol l$^{-1}$ HNO$_3$ and 0.5 ml of 30% v/v H$_2$O$_2$ can efficiently digest the same amount of vegetation sample [15,22].

The reduction of both sample and reagent volumes is a trend in instrumental analysis. This is useful in avoiding chemical attack of equipment parts, such as the nebulizer, nebulization chamber and torch in ICP-OES, and in minimizing the production of residue. In addition, depending on the plasma gas flow rate, a quartz torch in axial configuration can be rapidly degraded by crystallization due to the deposition of salts formed from samples containing high amounts of dissolved solids. Thus, sample digestion must suit the requirements of the analytical technique employed for measurement, and dilute acid solutions are an attractive alternative in performing sample digestion. In addition, water is a superb dipole molecule suited to efficient conversion of microwave energy, and the use of dilute solutions could imply better efficiency of microwave heating [23].

Visual observation of digestates showed that oxidant mixtures containing H$_2$O$_2$ produced colorless solutions without any organic solid residues. For samples digested with water instead of H$_2$O$_2$, digestates were yellow and solid residues remained in suspension.

Temperature and pressure curves were similar for samples digested using the same heating program and oxidant mixtures containing H$_2$O$_2$ with different amounts of HNO$_3$ (Fig. 1).

The similar behavior of temperature and pressure curves for all oxidant mixtures tested suggests that changes in RCC for digestates would possibly be produced by different acid concentrations. To check this hypothesis, RCC values were determined in all samples digested using different oxidant mixtures and the same heating program. Table 2 shows RCC values obtained for each digestion condition.

According to the RCC data, the use of more concentrated HNO$_3$ solutions led to slightly better oxidation of the organic matter (Table 2), showing, as expected, that the amount of acid influences the oxidation process [4]. However, efficient oxidation was also reached using dilute solutions of nitric acid, and the concentration of analytes determined using ICP-OES did not differ appreciably in all.
digestates, indicating that matrix interference was not a factor (Table 3). This implies that RCC does not affect ICP-OES measurements, even when using axial view configuration, which has also been reported by Gouveia et al. [19]. As previously mentioned, the possibility of using dilute solutions of HNO₃ improved the ICP-OES performance, avoiding wear of equipment components in contact with the dilute digestates.

In spite of slightly similar RCC values determined for all digestion conditions, different organic compounds were generated when using different HNO₃ concentrations. As discussed later, spectra obtained by NMR showed different types of aro-
Table 3
Analyte concentration in samples digested with 2 ml of HNO$_3$ (2 mol l$^{-1}$) plus 1 ml of H$_2$O$_2$ (30% v/v), $n=3$

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte concentration (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ba</td>
</tr>
<tr>
<td>Spinach leaves (NIST 1570a)</td>
<td></td>
</tr>
<tr>
<td>Certified</td>
<td>$^{\ast}$</td>
</tr>
<tr>
<td>Found</td>
<td>7.5 ± 3.0</td>
</tr>
<tr>
<td>Corn bran (NIST 8433)</td>
<td></td>
</tr>
<tr>
<td>Certified</td>
<td>2.40 ± 0.52</td>
</tr>
<tr>
<td>Found</td>
<td>2.35 ± 0.40</td>
</tr>
<tr>
<td>Mango leaves</td>
<td>4278 ± 163</td>
</tr>
<tr>
<td>Citrus leaves</td>
<td>4613 ± 94</td>
</tr>
<tr>
<td>$P$. regnellii</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>$P$. guenoarum</td>
<td>30.0 ± 0.3</td>
</tr>
<tr>
<td>$P$. glaucescens</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>$P$. notatum</td>
<td>107 ± 1</td>
</tr>
</tbody>
</table>

$^a$ Certified value not available.

$^b$ Non-certified concentration.
mamic and aliphatic organic compounds generated for each digestion condition.

3.2. Residual acidity in digestates

The amount of residual acid in each solution after digestion was determined by acid–base titration, with the results shown in Fig. 2. As expected, it can be observed that more concentrated solutions led to digestates containing a higher concentration of residual acid. On the other hand, consumption of the acid during the digestion process varied from 56.7 to 12.0% for 14 and 2 mol l⁻¹ HNO₃ solutions, respectively. The higher consumption of acid when using up to 5 mol l⁻¹ HNO₃ solution can be partially explained, considering that RCC values showed a slight decrease for more concentrated solutions (Table 2). However, these differences were not very pronounced and other processes could be occurring. It should be mentioned that all closed vessels were cooled down during 30 min in flowing water at room temperature after microwave heating. For digestates obtained from concentrated acid solutions, intense evolution of nitrogen oxides was observed, even after this cooling step. This could explain the highest consumption of nitric acid when using concentrated solutions. In addition, ¹H NMR spectra obtained for the digestates showed that residual organic compounds generated during digestion are comparatively more complex when using more concentrated acid solutions.

Fig. 3 shows a total view of NMR spectra of a sample of _P. notatum_ digested with the lowest and the highest concentrations of oxidant mixtures tested. It is possible to observe that the spectrum obtained after digestion with 2 mol l⁻¹ HNO₃ is simpler than that obtained with 14 mol l⁻¹ HNO₃. A strong signal at δ 5.86 ppm, present with similar intensity, can be observed in all ¹H NMR spectra. In the ¹³C NMR spectrum, a peak at δ 92.6 ppm (CH) was observed, suggesting aliphatic nitro-compounds. More NMR studies are necessary to elucidate the compound classes accurately. However, other conclusions can be drawn from the spectra:
Figs. 4 and 5 show NMR spectra obtained for aromatic ($\delta$ 7.0–10.5 ppm) and aliphatic ($\delta$ 0.5–3.0 ppm) regions, respectively. It is evident that the spectrum for which 14 mol l$^{-1}$ HNO$_3$ had been used presents a wider variety of organic compounds, such as nitro-derivative aromatic compounds, and a higher amount of aromatic and aliphatic compounds. In addition, it can be
observed that aliphatic organic residues are present in higher concentrations than aromatic organic residues, indicating that oxidative processes gradually convert complex organic molecules in simpler ones. Since concentrated nitric acid at high temperatures shows stronger oxidative power than dilute solutions, the former solution acts more randomly towards the breaking of chemical bonds.
and generates a plethora of organic residues. On the other hand, dilute nitric acid solutions do not have strong oxidative action, but at high temperatures exhibit effective digestion action, as demonstrated.

In the 14 mol $\text{l}^{-1}$ digestate residues, small peaks, probably carbinolic hydrogens, are present in the $\delta$ 4.0–4.7 ppm region. With a reduction in concentration, the intensity and variety of these signals were also reduced, until they practically

Fig. 6. $^1$H NMR spectra of the organic residue of different digestates, $\delta$ 4.0–4.7 ppm (carbinolic hydrogens).
disappeared when 2.0 mol l⁻¹ acid was used (Fig. 6).

4. Conclusions

It was demonstrated here that plant materials can be efficiently acid-digested in a closed-vessel microwave system with the use of dilute acids. The high temperature (225 °C) reached at high pressure (30 bar) generated an oxidative reaction medium, even at nitric acid concentrations as dilute as 2.0 mol l⁻¹. Spectra generated by NMR showed different classes of organic residues, depending on the concentration of nitric acid, even when the residual carbon content did not differ appreciably. Data obtained by NMR allowed a better understanding of operative processes during microwave-assisted acid digestion in closed vessels.

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