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# Evaluation of oxygen pressurized microwave-assisted digestion of botanical materials using diluted nitric acid

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## ABSTRACT

The feasibility of diluted nitric acid solutions for microwave-assisted decomposition of botanical samples in closed vessels was evaluated. Oxygen pressurized atmosphere was used to improve the digestion efficiency and Al, Ca, K, Fe, Mg and Na were determined in digests by inductively coupled plasma optical emission spectrometry (ICP OES). Efficiency of digestion was evaluated taking into account the residual carbon content (RCC) and residual acidity in digests. Samples were digested using nitric acid solutions (2, 3, 7, and 14 $\mathrm{mol\,L^{-1}}$ $HNO_3$) and the effect of gas phase composition inside the reaction vessels by purging the vessel with Ar (inert atmosphere, 1 bar), air (20% of oxygen, 1 bar) and pure $O_2$ (100% of oxygen, 1 bar) was evaluated. The influence of oxygen pressure was studied using pressures of 5, 10, 15 and 20 bar. It was demonstrated that a diluted nitric acid solution as low as 3 $\mathrm{mol\,L^{-1}}$ was suitable for an efficient digestion of sample masses up to 500 mg of botanical samples using 5 bar of oxygen pressure. The residual acidities in final digests were lower than 45% in relation to the initial amount of acid used for digestion (equivalent to 1.3 $\mathrm{mol\,L^{-1}}$ $HNO_3$). The accuracy of the proposed procedure was evaluated using certified reference materials of olive leaves, apple leaves, peach leaves and pine needles. Using the optimized conditions for sample digestion, the results obtained were in agreement with certified values. The limit of quantification was improved up to a factor of 14.5 times for the analytes evaluated. In addition, the proposed procedure was in agreement with the recommendations of the green chemistry once it was possible to obtain relatively high digestion efficiency ($RCC < 5\%$) using only diluted $HNO_3$, which is important to minimize the generation of laboratory residues.

## 1. Introduction

The determination of metals in botanical samples often requires a previous digestion step before analyte determination [1,2]. Closed vessels have been currently used in order to improve the oxidation efficiency and also to reduce the time of digestion. Although digestion using closed vessels may also employ conventional heating, most of the recent applications have been performed using microwave radiation in view of the relatively shorter time involved [3–5]. The possibility of working at high pressures in closed vessels causes an increase of the oxidant properties of nitric acid and for this condition the digestion of samples containing high content of organics can be carried out without addition of auxiliary reagents [6–8]. However, for samples that present high content of silica and organic compounds (e.g., botanical samples) problems related to poor recoveries have been observed, mainly for elements associated with silicon present in plants such as Al and Fe [9]. Therefore, the additions of auxiliary reagents, such as $H_2SO_4$, $H_2O_2$, $H_3BO_3$, HF and $HBF_4$, are frequently necessary for a complete release of analyte to solution [9–11].

Nevertheless, the addition of auxiliary reagents for digestion increases the risk of contamination and may result in digests with high salinity and acidity that could not be suitable for conventional sample introduction devices used in some atomic spectrometry techniques [9]. In addition, the use of concentrated reagents is always hazardous, it requires dilution of the digests before the determination step and can generate high volumes of concentrated acids as effluents. In this way, the use of diluted acid solutions have been mainly reported for procedures employing microwave [12,13] and ultrasound energy [14,15] in order to obtain an improvement during extraction. This possibility of using diluted acid was observed during the use of microwave-assisted extraction for metal

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determination by ICP OES in raft mussels samples (up to 500 mg). Recoveries higher than 95% were obtained using 15 mL of a mixture of diluted $HNO_3$, HCl and $H_2O_2$ (2.5 and 3.0 mol $L^{-1}$ and 0.5%, respectively) for Ba, Cd, Cr, Cu, Fe, Mn, Pb, Sn, V and Zn determination. Quantitative recovery was obtained for Al only using a mixture of diluted $HNO_3$, HF and $H_2O_2$ (2.5 and 3.0 mol $L^{-1}$ and 0.5%, respectively) [16]. Microwave-assisted extraction using diluted solutions (10 mL of $HNO_3$ and HCl, 5–20%) was also used for Se determination by electrothermal atomic absorption spectrometry in medicinal plants (up to 200 mg). It was observed that under optimized conditions the results obtained for CRM samples showed agreement higher than 95% [17]. In the same way, procedures using ultrasound energy can be also employed to assist metal leaching using diluted solutions. It was observed during determination of As, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, V and Zn in seaweed sample (up to 200 mg) using 7 mL of diluted solution of $HNO_3$, HCl and $H_2O_2$ (3.7, 3.0 and 3.0 mol $L^{-1}$, respectively) [18]. Determination of Cd, Cu and Zn in fish and mussel samples (up to 500 mg) by flame and graphite furnace atomic absorption spectrometry has been performed after ultrasound-assisted extraction procedure. Diluted solutions (6 mL) of $HNO_3$, HCl and $H_2O_2$ (4.0, 4.0 and 0.5 mol $L^{-1}$, respectively) were used and recoveries from 92 to 114% and from 88 to 103% were obtained for metal determination in fish and mussel samples, respectively [19].

In the same way, digestion procedure can be also performed using diluted acid solutions. Digestion procedures can minimize some drawbacks related to extraction procedure such as influence of particle size, the lack of accuracy for some elements and the complete dissolution of the analytes that must be ensured [12,14,15,18]. Performing reactions at high pressure and temperature may allow a reduction of the acid concentration without decreasing the efficiency of digestion. The feasibility of using diluted solutions containing lower concentrations of nitric acid has been experimentally demonstrated [20–22]. The efficiency of using diluted acids is a result of the temperature gradient inside the reaction vessel during the initial steps of sample digestion and due to the presence of a rich oxygen atmosphere inside the closed-vessel [22,23]. An important aspect is related to the poor absorption of microwave radiation by the gas phase that, despite this aspect, it can improve the oxidation processes. The combination of both effects results that reaction products can be oxidized also at the upper atmosphere of the reaction vessel and further reabsorbed in the liquid phase. Therefore, the use of oxygen atmosphere together with diluted nitric acid leads to a regeneration of nitric acid and to an improvement on digestion efficiency of samples [23].

Considering that sample matrix has a great influence on digestion efficiency and analyte recoveries the microwave-assisted digestion in closed vessels using diluted nitric acid within oxygen pressurized atmosphere was evaluated in the present work for botanical materials. The determination of Ca, K, Mg, Na, Al and Fe, elements that must be currently determined in plants, was performed by inductively coupled optical emission spectrometry (ICP OES) in oregano leaves and in botanical certified reference materials (CRM). In addition, it was tried to reduce as much as possible the amount of nitric acid needed for the digestion process in order to minimize the blank values and also decrease the consumption of reagents and the generation of laboratory residues.

## 2. Experimental

### 2.1. Instrumentation

A microwave oven (Multiwave 3000 microwave sample preparation system, Anton Paar, Graz, Austria) equipped with eight high-pressure quartz vessels was used in the experiments. The internal volume of vessels was 80 mL and the maximum operational temperature and pressure was set at 280 °C and 80 bar, respectively. Pressure and temperature were monitored in each vessel for all the runs.

**Table 1**
ICP OES operational parameters for determination of C, Al, Ca, Fe, K, Mg and Na.

| Parameter | ICP OES |
|---|---|
| Radio-frequency power (W) | 1600 |
| Plasma gas flow rate (L $min^{-1}$) | 14.0 |
| Auxiliary gas flow rate (L $min^{-1}$) | 1.0 |
| Nebulizer gas flow rate (L $min^{-1}$) | 0.85 |
| Spray chamber | Double pass, Scott type |
| Nebulizer | Cross-flow |
| Observation view | Axial |
| Emission line (nm) | |
| C (I) | 193.091 |
| Al (I) | 167.078 |
| Ca (II) | 317.933 |
| Fe (I) | 238.204 |
| K (I) | 766.491 |
| Mg (I) | 285.213 |
| Na (I) | 589.592 |

Analytes were determined by ICP OES using an axial view configuration spectrometer (Spectro Ciros CCD, Spectro Analytical Instruments, Kleve, Germany). Nebulization was performed through a cross-flow nebulizer coupled to a Scott double pass type nebulization chamber. Plasma operating conditions and selected wavelengths used for analytes determination are listed in Table 1, and they were used as recommended by the instrument manufacturer [24]. For the determination of residual carbon content (RCC, mg C/100 mg of sample) [25] digested solutions were determined by ICP OES [26]. In order to remove the volatile carbon compounds before RCC determination, samples were previously sonicated with a ultrasonic probe [27] (VCX 130 PB, 130 W, 20 kHz, Sonics and Materials Inc., Newton, CT, USA). Argon (99.996%, White Martins-Praxair, São Paulo, SP, Brazil) was used in ICP OES determinations for plasma generation, nebulization, auxiliary gas and also for digestion performed under inert atmosphere. Oxygen (99.9991%, White Martins-Praxair) was used as reagent in digestions performed under oxygen pressure.

Results for residual acidity were obtained using a titration system (Titrando 836, Metrohm, Herisau, Switzerland) equipped with a magnetic stirrer (module 803 Ti Stand), 20 mL burette (Dosino 800) and pH electrode (LL Electrode plus, model 6.0262.100).

### 2.2. Samples, reagents and standards

Preliminary experiments were carried out using the leaves of *Origanum majorana* (oregano) as sample, which was purchased in a local market. This sample was dried using an oven (model 400/2ND, Nova Ética, Vargem Grande Paulista, SP, Brazil) and ground using a cryogenic mill (Spex Certi Prep, model 6750, Metuchen, NJ, USA). The final particle diameter was below 102 μm. Accuracy was evaluated using a CRM of trace elements in olive leaves (BCR 62) produced by Community Bureau of Reference (BCR, Brussels, Belgium) and by using standard reference materials (SRM) of apple leaves (SRM NIST 1515), peach leaves (SRM NIST 1547) and trace elements in pine needles (SRM NIST 1575a), produced by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Samples were accurately weighed using an analytical balance (model AY 220, max. 220 g, 0.1 mg of resolution, Shimadzu, Kyoto, Japan).

Distilled-deionized water (Milli-Q, 18.2 MΩ cm, Millipore, Billerica, MA, USA) and analytical-grade nitric acid (Merck, Darmstadt, Germany) were used to prepare samples and standards. Carbon reference solutions used to external calibration for RCC determination were prepared by dissolution of citric acid (Merck) in water (25–500 mg $L^{-1}$ of C). Yttrium (1.0 mg $L^{-1}$, Spex CertPrep, Metuchen, New Jersey, USA) was used as internal standard in all samples, blanks and reference solutions for RCC determination. Metals determination by ICP OES was performed with external calibration using analytical solutions from 1.0 to 100 μg $L^{-1}$, prepared in 0.7 mol $L^{-1}$ $HNO_3$ by appropriate dilution of the multi-element stock solution (SCP33MS, SCP Science, Quebec, Canada). A 0.1 mol $L^{-1}$ KOH (Merck) solution was used to residual acidity determination. Glass and quartz material were soaked in 1.4 mol $L^{-1}$ $HNO_3$ for 24 h and further washed with water before use.

### 2.3. Microwave-assisted acid digestion

Sample aliquots of 500 mg were directly inserted into the closed quartz vessels. Digestion efficiency using nitric acid solutions (6 mL) was evaluated in the following concentrations: 2, 3, 7, and 14 mol $L^{-1}$. After closing and capping the rotor, an oxygen flow was passed inside the vessel (1 bar of pressure, atmosphere with 100% of $O_2$) or vessels were pressurized with 5, 10, 15 and 20 bar of oxygen. The introduction of gases inside the vessels was performed using the valve originally designed for pressure release after conventional acid sample digestion. The same procedure was carried out without oxygen pressure (1 bar of pressure of atmospheric air) and, additionally, with inert atmosphere (1 bar of pressure, atmosphere with 100% of Ar). Then, rotor was placed inside the oven, and microwave-heating program was started by applying (i) 1000 W with a ramp of 5 min, (ii) 1000 W for 10 min, and (iii) 0 W for 20 min (cooling step) [28]. After digestion, the pressure of each vessel was carefully released. In this work, each run was performed using four vessels. The resulting solutions were transferred to 30 mL polypropylene vials and diluted up to the mark with water. Cleaning of digestion vessels was carried out with 6 mL of concentrated $HNO_3$ in the microwave oven at 1000 W for 10 min and 0 W for 20 min for cooling. Final digests were analyzed by ICP OES for both RCC and analyte determination and residual acidity were evaluated by titration. All statistical calculations were performed using *t* test (GraphPad InStat Software Inc, Version 3.00, 1997). A significance level of $P < 0.05$ was chosen for all comparisons.

## 3. Results and discussion

### 3.1. Evaluation of digestion efficiency

Preliminary experiments were performed for oregano leaves samples in order to evaluate the influence of oxygen pressure on sample digestion by the use of nitric acid solutions with and without oxygen. A pressure of 20 bar of oxygen was arbitrarily selected and due to safety reasons higher pressures were not tested in this work. Sample masses of 500 mg were used and RCC and residual acidity were determined in final digests. Results for digestion of 500 mg of oregano leaves are shown in Fig. 1. Without oxygen pressure, i.e., air atmosphere inside the vessel, lower RCC (<3%) was obtained only if nitric acid solution containing at least 7 mol $L^{-1}$ was used and, for this case, clear solutions were always obtained. However, using 3 mol $L^{-1}$ $HNO_3$, final digests presented a yellow color and RCC values were higher, about 16%. When using 2 mol $L^{-1}$ $HNO_3$ the digestion was not effective and solid residues remained as suspended particles with a deep brown colored aspect and RCC values as high as 47.6% were obtained.

Using 20 bar of oxygen no difference was observed in final digests for both concentrated and 7 mol $L^{-1}$ nitric acid. Values of RCC lower than 4% were obtained for both conditions and these results were compared with results without $O_2$ pressure. In this case, the presence of $O_2$ had no detectable effect and these results may be explained by the high oxidant environment generated by elevated concentrations of nitric acid even without $O_2$. However, a more effective digestion was obtained with 20 bar of oxygen pressure for nitric acid solutions containing 3 mol $L^{-1}$ (RCC = 5%), and final digests presented a colorless solution aspect. Therefore, it was possible to reduce the nitric acid concentration about 2.3 times but still obtaining a similar digestion efficiency when compared with the use of more concentrated nitric acid solution. Using more diluted solutions of $HNO_3$ the efficiency was not the same. After digestion performed using 2 mol $L^{-1}$ $HNO_3$, final digests present a colorless solution and solid residues as suspended particles were not observed. However, RCC values were still higher, around 15%, when they were compared with those using more concentrated nitric acid solutions (RCC lower than 5%). When using lower $HNO_3$ concentrations the effect of $O_2$ decreased and this effect may be explained by the low oxidation power of a solution containing 1 mol $L^{-1}$ $HNO_3$ even in the presence of 20 bar of oxygen.

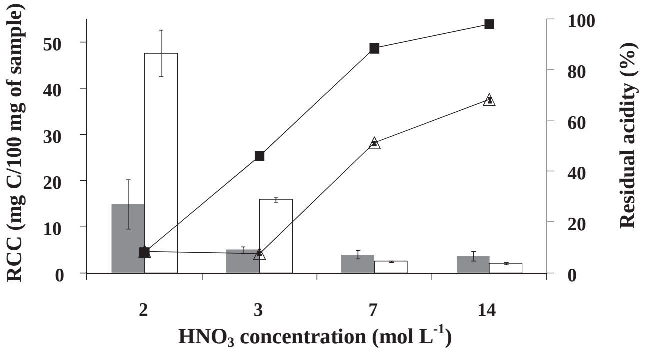

**Fig. 1.** Effect of nitric acid concentration on digestion of 500 mg of oregano sample; effectiveness of organic matter digestion performed under oxygen pressure (gray bars) and without oxygen pressure (white bars). Lines represent the residual acidity obtained from digestion performed under oxygen pressure (-■-) and without oxygen pressure (-△-). Errors bars represent the standard deviation ($n = 3$).

Therefore, even using an acid solution as diluted as a 3 mol $L^{-1}$ $HNO_3$ to digest 500 mg of botanical sample the RCC values were always lower than 5% using 20 bar of oxygen and this condition was used for subsequent tests.

### 3.2. Residual acidity

As reported in a previous study [23], the oxidant action of nitric acid may be improved if a regenerating process occurs, which is mainly dependent on the amount of oxygen available in gas phase during the oxidation of the organic matter. This reaction could be effective with diluted nitric acid if digestion vessels were pressurized with oxygen. This hypothesis was reinforced by the results obtained for residual acidity showed in Fig. 1. The residual acidity obtained when digestion was performed without oxygen pressure presented values ranged from 68% (14 mol $L^{-1}$ $HNO_3$) to 8% (3 and 2 mol $L^{-1}$ $HNO_3$). The evaluation of residual acidity in final digest performed under oxygen pressure presented values from 98% (14 mol $L^{-1}$ $HNO_3$) to 8% (2 mol $L^{-1}$ $HNO_3$). It may be seem that residual acidity in the same reaction conditions was higher with tests using oxygen pressure and this fact may be explained due to the occurrence of the proposed regeneration process [20,23]. Besides, residual acidity, obtained when digestion was performed using 2 and 3 mol $L^{-1}$ $HNO_3$ without oxygen pressure (Fig. 1, line with white triangle), presented low values of residual acidity (8%). However, a lower digestion efficiency was found and a yellowish color in digests with solid residues remaining as suspended particles was obtained. The same behavior was observed for digestion performed with 2 mol $L^{-1}$ $HNO_3$ with oxygen pressure. An effi-

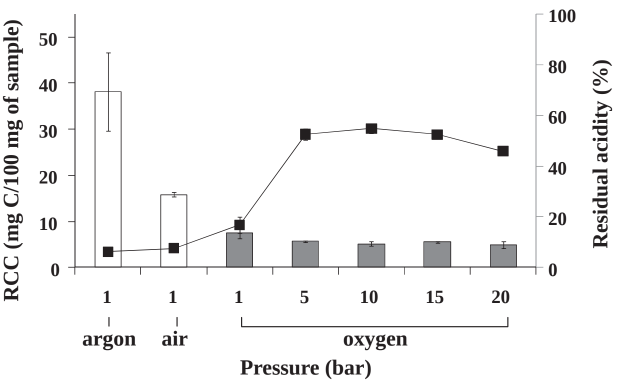

**Fig. 2.** Influence of gas phase inside the reaction vessel. Effect of oxygen pressure (gray bars) and argon or atmospheric air (white bars) on digestion efficiency of 500 mg of botanical sample. Digestion performed using 3 mol $L^{-1}$ $HNO_3$. Lines represent the residual acidity obtained from digestion performed with 3 mol $L^{-1}$ $HNO_3$ (-■-). Errors bars represent the standard deviation ($n = 3$).

cient digestion (RCC = 5%) was observed using 3 mol $L^{-1}$ $HNO_3$ with oxygen pressure and 7 mol $L^{-1}$ $HNO_3$ without oxygen pressure. In these conditions, residual acidity was around 45% (equivalent to 1.3 mol $L^{-1}$ $HNO_3$) and RCC values were lower than 5%.

Moreover, after digestion it was possible to observe final digests with colorless gas phase inside the reaction vessel when digestion was performed under oxygen pressure. This result may be due to the absence of $NO_2$ in gas phase, probably owing to the regeneration process of nitric acid promoted by oxygen. The opposite behavior was observed in final digests obtained from experiments performed without oxygen pressure, where brown fumes of $NO_2$ were always observed inside the reaction vessels.

### 3.3. *Influence of the gas phase inside the reaction vessel*

As previously discussed [23], the $HNO_3$ regeneration process was not dependent on the initial pressure of digestion procedure. Thus, for evaluating the effect of gas phase inside the digestion vessel on the efficiency of digestion, oregano samples were digested under different oxygen pressures of oxygen, ranging from 1 to 20 bar, and also with atmospheric air (1 bar of air) and with argon (1 bar of argon). For this study a 3 mol $L^{-1}$ $HNO_3$ solution was used to digest 500 mg of sample.

As it can be seen in Fig. 2, for sample masses of 500 mg and oxygen pressure ranging from 5 to 20 bar using 3 mol $L^{-1}$ $HNO_3$ the efficiency of digestion was quite similar (RCC was always lower than 5.5%, Fig. 2, gray bars) and final digests presented a clear aspect without solid residues in suspension. Besides, the residual acidity for both conditions presented similar values (53, 55, 53 and 45% (equivalent to 1.3 mol $L^{-1}$ $HNO_3$) for oxygen pressure of 5, 10, 15 and 20 bar, respectively), which represents an amount of oxygen enough to maintain the regeneration process of $HNO_3$, as it can be observed in Fig. 2 (line with black square). However, when digestion vessel was purged with oxygen (atmosphere with 100% of oxygen, 1 bar), final digests also remain as clear solutions but digestion efficiency was slightly lower with RCC about 7.5%. Using this condition it was observed a drastic decrease in residual acidity (17%), which could be considered relatively low to assure a complete organic matter digestion. This fact may be related to an insufficient amount of oxygen in gas phase to promote the regeneration of nitric acid.

The influence of gas phase inside the reaction vessel on digestion efficiency and residual acidity was also evaluated with tests without pure oxygen pressure (Fig. 2). When digestion was performed with only atmospheric air inside reaction vessel (1 bar, about 20% of $O_2$), the RCC of final digests was about 16% (Fig. 2, white bar) and solution presented a yellowish aspect. A low value of residual acidity was found (8%) and a possible explanation could be related to the low amount of oxygen present in atmospheric air that could not be enough to regeneration of $HNO_3$. Additional tests were performed using diluted nitric acid under an inert gas pressure. In this case, digestion vessel was purged with argon (atmosphere with 100% of argon, 1 bar). Digestion was performed with 500 mg of sample mass using 3 mol $L^{-1}$ $HNO_3$. It was observed a yellowish color in digests and residues remaining as suspended particles (values for RCC were higher than 38% after removing particles by centrifugation). These values were appreciably higher than those obtained when using the same pressure but with oxygen (colorless digests without solids in suspension, RCC = 7.5%) instead argon. In addition, the residual acidity found in the procedure performed with inert gas phase was lower than that when working with 1 bar of oxygen (residual acidity = 6 and 17%, respectively) which confirms the effect of oxygen on digestion efficiency and the possible regeneration of $HNO_3$. Therefore, in the range of oxygen pressure evaluated in this work, no significant changes were observed at pressures higher than 5 bar in relation to RCC and residual acidity and this pressure of oxygen was selected for subsequent experiments.

### 3.4. *Determination of Al, Ca, K, Fe, Mg and Na by ICP OES in certified reference materials and botanical sample*

Accuracy was evaluated by using CRMs of olive leaves, apple leaves, peach leaves and pine needles. Digestions of CRM samples and oregano leaves were performed using optimized conditions (500 mg of sample mass, 3 mol $L^{-1}$ $HNO_3$ solutions and 5 bar of $O_2$). Results obtained for Al, Ca, K, Fe, Mg and Na in CRM materials using the proposed digestion procedure presented a good agreement (better than 95%, $P < 0.05$) with certified values as it may be seen in Table 2. It is important to mention that Al and Fe are usually found as silicon-bound compounds in plant matrices. In dried samples silicon content generally ranges from 0.2 to 30 mg $g^{-1}$ and, in some cases, low recoveries may be observed if auxiliary reagents (e.g., HF) were not used [9]. However, in the present work it was observed that results obtained for CRMs of botanical samples presented quantitative recoveries for all the analytes including Al and Fe. This fact confirms that the proposed procedure using diluted nitric acid under oxygen pressurized atmosphere was suitable for

**Table 2**

Elements determined in botanical CRMs by ICP OES (mean and standard deviation in μg $g^{-1}$, $n = 5$). Digestion conditions: 3 mol $L^{-1}$ $HNO_3$ and 5 bar $O_2$.

| Analyte | SRM NIST 1515 (apple leaves) | | SRM NIST 1547 (peach leaves) | | SRM NIST 1575a (pine needle) | | CRM BCR 62 (olive leaves) | | Oregano leaves |
|---|---|---|---|---|---|---|---|---|---|
| | Found | Certified | Found | Certified | Found | Certified | Found | Certified | Found |
| Al | 288 ± 2 | 286 ± 9 | 255 ± 7 | 249 ± 8 | 577 ± 9 | 580 ± 30 | 469 ± 18 | 450 ± 20 | 482 ± 1 |
| Ca | 15292 ± 96 | 15260 ± 150 | 15626 ± 23 | 15600 ± 200 | 2457 ± 49 | 2500 ± 100 | 17273 ± 158 | – | 12608 ± 286 |
| Fe | 79.1 ± 4.3 | 83 ± 5 | 215 ± 3 | 218 ± 14 | 46.2 ± 0.8 | 46 ± 2 | 348 ± 17 | – | 292 ± 15 |
| K | 16237 ± 120 | 16100 ± 200 | 24439 ± 213 | 24300 ± 300 | 4082 ± 98 | 4170 ± 70 | 4403 ± 278 | – | 25560 ± 579 |
| Mg | 2706 ± 43 | 2710 ± 80 | 4339 ± 20 | 4320 ± 80 | 1027 ± 87 | 1060 ± 170[a] | 1003 ± 44 | – | 2174 ± 34 |
| Na | 25.1 ± 4.3 | 24.4 ± 1.2 | 24.3 ± 1.8 | 24 ± 2 | 59.8 ± 3.6 | 63 ± 1[a] | 108 ± 8 | – | 103 ± 7 |

[a] Informed values.

**Table 3**
Limits of quantification (LOQ) for 500 mg of botanical sample digested by conventional wet digestion (digestion using $HNO_3$ 14 mol $L^{-1}$) and by the proposed procedure (digestion using $HNO_3$ 3 mol $L^{-1}$ with 5 bar of oxygen pressure).

| Analyte | LOQ, μg $g^{-1}$ (10$\sigma$) | |
|---|---|---|
| | Conventional wet digestion | Proposed procedure |
| Al | 0.20 | 0.05 |
| Ca | 4.19 | 0.29 |
| Fe | 0.88 | 0.82 |
| K | 0.33 | 0.20 |
| Mg | 0.09 | 0.08 |
| Na | 0.43 | 0.08 |

determination of these metals in botanical samples. In this way, the same digestion procedure was used for Al, Ca, K, Fe, Mg and Na determination in oregano sample and results are also shown in Table 2.

The use of diluted nitric acid and vessels pressurized with oxygen resulted in low blank values that could have impact on the limit of quantification (LOQ) of some elements. In this work, this effect could be observed for LOQ from digestion of 500 mg of sample mass (Table 3). The LOQ was similar for Fe, K and Mg for both digestion procedures (using concentrated and 3 mol $L^{-1}$ $HNO_3$ under oxygen pressurized atmosphere). However, using the proposed digestion procedure (3 mol $L^{-1}$ $HNO_3$ under 5 bar of $O_2$) the LOQs for Al, Ca and Na were 4, 14.5 and 5 times lower than the LOQs obtained by the digestion procedure with concentrated nitric acid conventionally used. This result could be explained by the use of more diluted nitric acid during proposed digestion procedure that allows a reduction in blank values and consequently in LOQs.

## 4. Conclusions

Experimental data suggesting the regeneration of $HNO_3$ could be explained by the oxygenated atmosphere that improved the effectiveness of digestion using diluted nitric acid solutions. In this way, the use of diluted nitric acid associated with oxygen pressurized atmosphere was proven to be feasible and strongly recommended for sample digestion, reducing the volume of the reagents and the amount of digestion residues. Using digestion vessels under oxygen rich atmosphere (1 bar of $O_2$) and with oxygen pressures up to 20 bar, it was possible to bring into solution sample masses of up to 500 mg with only an equivalent amount of 1.28 mL of concentrated nitric acid. For digestion performed in the same conditions, but without oxygen pressure, 3 mL of concentrated nitric acid were necessary. These results represent a decrease of about 2.3-times of the volume of concentrated nitric acid. In addition, a considerable reduction in residual acidity (about 60%) was observed in relation to the initial nitric acid solution used during sample digestion, which represents a minimal amount of nitric acid that was not used during organic matter oxidation process. Another advantage is related to the use of only diluted nitric acid without the need of others auxiliary reagents. Good agreements were obtained for CRMs from four different matrices of botanical materials, mainly for Al and Fe that were commonly found as silicate-bound elements. It is also important to mention that these aspects are in agreement with the recommendations of green chemistry [29–31]. Finally, when digestion was performed under optimized condition (sample mass of 500 mg, 3 mol $L^{-1}$ $HNO_3$ and 5 bar of oxygen pressure) blank values were decreased and, consequently, the LOQ values for Al, Ca and Na that were reduced 4, 14.5 and 5 times, respectively.

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## References

[1] Z. Mester, R. Sturgeon, Sample Preparation for Trace Element Analysis, Elsevier, Amsterdam, 2003.
[2] M. Burguera, J.L. Burguera, Anal. Chim. Acta B 366 (1998) 63–80.
[3] F.A. Duarte, J.S.F. Pereira, J.S. Barin, M.F. Mesko, V.L. Dressler, E.M.M. Flores, G. Knapp, J. Anal. At. Spectrom. 24 (2009) 224–227.
[4] K. Srogi, Anal. Lett. 40 (2007) 199–232.
[5] M. Soylak, M. Tuzen, I. Narin, H. Sari, J. Food Drug Anal. 12 (2004) 254–258.
[6] E.M.M. Flores, A.P.F. Saidelles, J.S. Barin, S.R. Mortari, A.F. Martins, J. Anal. At. Spectrom. 16 (2001) 1419–1423.
[7] H.M. Kingston, L.B. Jassie, Introduction to Microwave Sample Preparation. Theory and Practice, American Chemical Society, Washington, 1988.
[8] M.A.Z. Arruda, Trends in Sample Preparation, Nova Science Publishers, New York, 2006.
[9] J. Sucharová, I. Suchara, Anal. Chim. Acta 576 (2006) 163–176.
[10] I. Lavilla, A.V. Filgueiras, C. Bendicho, J. Agric. Food Chem. 47 (1999) 5072–5077.
[11] A.A. Momen, G.A. Zachariadis, A.N. Anthemidis, J.A. Stratis, Microchim. Acta 160 (2008) 397–403.
[12] F.E. Smith, E.A. Arsenault, Talanta 43 (1996) 1207–1268.
[13] J.A. Nóbrega, L.C. Trevizan, G.C.L. Araújo, A.R.A. Nogueira, Spectrochim. Acta Part B 57 (2002) 1855–1876.
[14] M.D. Luque de Castro, F. Priego Capote, Analytical Applications of Ultrasound, Elsevier, Oxford, 2007.
[15] F. Priego Capote, M.D. Luque de Castro, Trends Anal. Chem. 23 (2004) 644–653.
[16] E.M. Seco-Gesto, A. Moreda-Piñeiro, A. Bermejo-Barreira, P. Bermejo-Barreira, Talanta 72 (2007) 1178–1185.
[17] N.F. Kolachi, T.G. Kazi, J.A. Baig, G.A. Kandhro, S. Khan, H.I. Afridi, S. Kumar, A.Q. Shah, J. AOAC Int. 93 (2010) 694–702.
[18] R. Domínguez-González, A. Moreda-Piñeiro, A. Bermejo-Barrera, P. Bermejo-Barrera, Talanta 66 (2005) 937–942.
[19] N. Manutsewee, W. Aeungmaitrepirom, P. Varanusupakul, A. Imyim, Food Chem. 101 (2007) 817–824.
[20] G.C.L. Araújo, M.H. Gonzalez, A.G. Ferreira, A.R.A. Nogueira, J.A. Nóbrega, Spectrochim. Acta Part B 57 (2002) 2121–2132.
[21] M.H. Gonzalez, G.B. Souza, P.V. Oliveira, L.A. Forato, J.A. Nóbrega, A.R.A. Nogueira, Talanta 79 (2009) 396–401.
[22] J.T. Castro, E.C. Santos, W.P.C. Santos, L.M. Costa, J.A. Nóbrega, M.G.A. Korn, Talanta 78 (2009) 1378–1382.
[23] C.A. Bizzi, E.M.M. Flores, R.S. Picoloto, J.S. Barin, J.A. Nóbrega, Anal. Methods 2 (2010) 734–738.
[24] Spectro Ciros CCD, software version 01/March 2003, Spectro Analytical Instruments GmbH & Co. KG: Kleve, Germany.
[25] A. Krushevska, R.M. Barnes, C.J. Amarasiriwaradena, H. Foner, L. Martines, J. Anal. At. Spectrom. 7 (1992) 845–850.
[26] S.T. Gouveia, F.V. Silva, L.M. Costa, A.R.A. Nogueira, J.A. Nóbrega, Anal. Chim. Acta 445 (2001) 269–275.
[27] E.M.M. Flores, M.F. Mesko, D.P. Moraes, J.S.F. Pereira, P.A. Mello, J.S. Barin, G. Knapp, Anal. Chem. 80 (2008) 1865–1870.
[28] Anton Paar GmbH, Multiwave 3000®, Microwave Sample Preparation System, Software version v1.27-Synt, Graz, Austria, 2003.
[29] F.R.P. Rocha, L.S.G. Teixeira, J.A. Nóbrega, Spectrosc. Lett. 42 (2009) 418–429.
[30] M.F. Mesko, P.A. Mello, C.A. Bizzi, V.L. Dressler, G. Knapp, E.M.M. Flores, Anal. Bioanal. Chem. 398 (2010) 1125–1131.
[31] M. Guardia, Trends Anal. Chem. 29 (2010) 577.