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Graphical and textural abstract

Food and biological samples were digested automatically in a high pressure (40 bar) flow digestion system with a large volume reactor (13.5 mL heated volume inside the microwave cavity).

High pressure microwave-assisted flow digestion system using a large volume reactor - Feasibility for further analysis by inductively coupled plasma -based techniques

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Abstract

A new high pressure (40 bar) continuous flow system with a large volume reactor (13.5 mL heated volume inside the microwave cavity) has been developed. The microwave-assisted digestion of the samples occurred in a coiled perfluoroalkoxy (PFA) tube reactor. As the mechanical stability of the PFA-tube is insufficient at the used digestion conditions (40 bar, >200 °C), it was placed inside an autoclave constructed from a thick-walled borosilicate tube and pressurized by nitrogen. Nitric acid and mixtures of HNO_3 with HCl and / or HF were used for sample digestion and no elevated blank levels caused by contamination with corrosion products from the flow digestion system were encountered. For glucose, glycine and phenylalanine a residual carbon content (RCC) of 2.3 ± 0.5, 37 ± 3 and 77.9 ± 0.7 % (mean ± standard deviation, n = 5), respectively, was obtained under optimized digestion conditions (500 W microwave power and 5.0 mL min^{-1} carrier flow rate). The accuracy of the method was evaluated using certified reference materials (NIST SRM 1577b, SRM 1515). The determined values were in good agreement with the certified ones using inductively coupled plasma optical emission spectrometry (ICP-OES) for analyte quantification. Moreover, a comparison between closed vessel microwave-assisted digestion and high pressure flow digestion was performed using several plant- an animal tissue samples. These materials were less finely ground than CRM's making slurry generation more difficult. Nevertheless, the element concentrations obtained by ICP-OES after flow digestion were in good agreement with those from closed vessel batch digestion.

Introduction

The microwave-assisted digestion is well known and widely applied in sample preparation for the determination of trace elements in various sample matrices. When performed in closed vessels analyte losses and contamination are avoided, a fast and efficient digestion is promoted and reagent consumption and waste generation is reduced. This technique is usually performed in batch mode, and therefore requires various handling steps like opening and closing the vessels or cleaning steps after each digestion. These procedures are time consuming and - despite the fast heating step – add significantly to the total time required for the digestion step. Moreover, these steps are very difficult to automate, making manual operation mandatory. In this respect, flow digestion is an attractive alternative as sample introduction, collection of the digested samples or dilution is easier to automate. Moreover, flow digestion systems can be easily interfaced to techniques for analyte quantification (e.g. inductively coupled plasma optical emission spectrometry – ICP-OES or inductively coupled plasma mass spectrometry – ICP-MS)[1-3].

However, flow digestion systems also have limitations: due to the low residence time of the sample in the heated region, the digestion efficiency is lower compared to batch mode digestion. Solid samples are introduced as slurries and must be sufficiently homogeneous to be representative for the bulk material. Despite these drawbacks, several flow digestion systems have been developed to make this sample preparation technique applicable to routine work. [1, 4]

Different types of microwave-assisted flow digestion systems have been described in literature. Based on the applied pressure during digestion, they can be broadly classified as ambient pressure[5-9], medium pressure (< 25 bar)[10-12] or high pressure (> 25 bar)[13-15] flow digestion systems. Another classification can be made based on the flow regime during digestion, distinguishing between continuous flow [5-7, 10-15] and stopped flow [8, 9, 16] systems. The main shortcoming of ambient and medium pressure systems is the lower digestion temperature, which – in combination with the short residence time inside the heated zone - causes inefficient digestion especially for solid samples. Digestion time and therefore the mineralization efficiency can be increased by applying the stopped flow principle. However, the sample throughput is thereby decreased. Working under high pressure conditions can ease digestion efficiency problems dramatically, as the digestion pressure is strongly correlated with the temperature inside the reactor. Higher digestion pressure is followed by higher reaction temperature that in turn accelerates the digestion process[3].

Few high pressure flow digestion systems have been reported in literature. Haiber and Berndt [15] developed a high pressure system operating at up to 360 °C and 300 bar pressure by using a resistively heated digestion tube made from Pt/Ir. Despite the very low RCC obtained, mixtures of nitric and hydrochloric acid drastically reduced the digestion tube lifetime.[17]

Pichler et al.[13] used a PTFE tube to form an inert reactor for sample decomposition. As the polymer cannot withstand a pressure of up to 35 bar at more than 200 °C it was coiled inside a pressurized autoclave. Thereby a pressure equilibrium between the digestion tubes inner and outer surface was attained. The digestion efficiency of this system was evaluated through digestion of glucose, glycine and phenylalanine. These substances can be considered as easy, moderate and difficult to digest, respectively. In this system the RCC was 1%, 15 % and 35 % for glucose, glycine and phenylalanine for solutions containing 9 g L^{-1} carbon in 6 mol L^{-1} nitric acid (8 min. digestion time). As a mono-mode microwave cavity was used, 110 W power at a flow rate of 0.6 mL min^{-1} sufficed to attain the reported RCC.

Wiltsche et al.[14] inserted a PFA tube into a coiled borosilicate glass tube, forming a N_2 pressurized helical reactor. This combination of a glass and polymeric material permitted to reach higher temperatures (ca. 230 °C) and pressures (40 bar). The digestion efficiency - expressed as residual carbon content (RCC) - of glucose in 6 mol L^{-1} nitric acid was 14%, when a flow rate of 2.0 mL min^{-1} and 800 W microwave power (multimode cavity) were used. However, unsatisfactory results were obtained for glycine as the RCC was 96%. The digestion efficiency was lower in this system due to the small reactor volume inside the microwave radiated zone. The authors reported that it was impossible to introduce more than three turns of PFTE tube in the coiled glass reactor due to the progressive increase of friction when the PTFE was put inside the glass tube.

The aim of this work was to develop a new, large volume reactor for high pressure continuous flow digestion that improves the digestion efficiency, permits to work with higher carrier flow-rates and thereby increases the sample throughput. ICP-OES was used for analyte quantification.

Experimental

Flow digestion system

A high pressure, continuous flow, pressure equilibrium flow digestion system[14] was used in this work. The main disadvantage of the previously developed instrument was the short

residence time of the sample inside the microwave cavity. Consequently a completely new pressure reactor design was developed (Fig 1) with a total digestion volume of 13.5 mL inside the microwave heated zone: the sample digestion was performed in a perfluoroalkoxy (PFA) tube (1.5 mm inner diameter, 2.5 mm outer diameter) tightly wound around a 300 mm long coil former (E in Fig 1) made from a PTFE tube (18 mm outer diameter; 8 mm inner diameter). The end of the digestion tube was returned through the coil former inner bore and left the microwave irradiated zone of the high pressure reactor through a hole in the lower end cap (F in Fig 1). The coil former with the digestion tube was placed inside a 28 mm ID precision pulled borosilicate glass tube with a wall thickness of 9.8 mm (K in Fig 1). This glass tube is rated for 50 bar pressure (the actual burst pressure is higher, because the rated value already includes a safety margin) and forms together with the two polyetheretherketone (PEEK) end caps (F and L in Fig 1) a pressurized autoclave. Two about 20 mm tick PEEK base plates (I and J in Fig 1) and four 16.4 mm diameter PEEK rods (G in Fig 1) held the end caps in place, when the glass tube was pressurized. Two stainless steel tubes connected the PFA digestion tube to the sample handling parts of the flow digestion system, that where arranged up- and downstream the microwave irradiated pressure reactor: Briefly, in this flow digestion system diluted nitric acid (0.2 mol L^{-1}) was continuously pumped through the system by an acid resistant all Ti HPLC pump (Fig 1 A; Knauer, Berlin, Germany). The samples, supplied by an autosampler (ASX-1400, Cetac, Omaha, NE, USA) were introduced into the high pressure stream through a sample loop connected to a 6-port valve (Fig 1 B; Knauer). By using a high precision dispenser (1-Channel MultiDispenser, ProLiquid, Germany) different sample volumes could be introduced into the flow digestion system. Moreover, each sample was embedded between two 2 mL segments of 6 mol L^{-1} nitric acid, to avoid dilution of the digestion solution by the carrier. The digestion took place in the aforementioned pressurized (40 bar) reactor, placed inside the multimode cavity of a commercial microwave digestion oven (Multiwave 3000, Anton Paar, Graz, Austria). A small but steady flow of nitrogen was introduced into the flow digestion system in a countercurrent stream with respect to the sample to remove acid vapors originating from diffusion of steam though the digestion tube. After leaving the microwave irradiated zone the digested samples were cooled down (Fig 1 O) and left the flow digestion setup through a restrictor capillary (Fig 1 Q). Details on the sample handling up- and downstream the pressurized reactor have been reported elsewhere.[14]

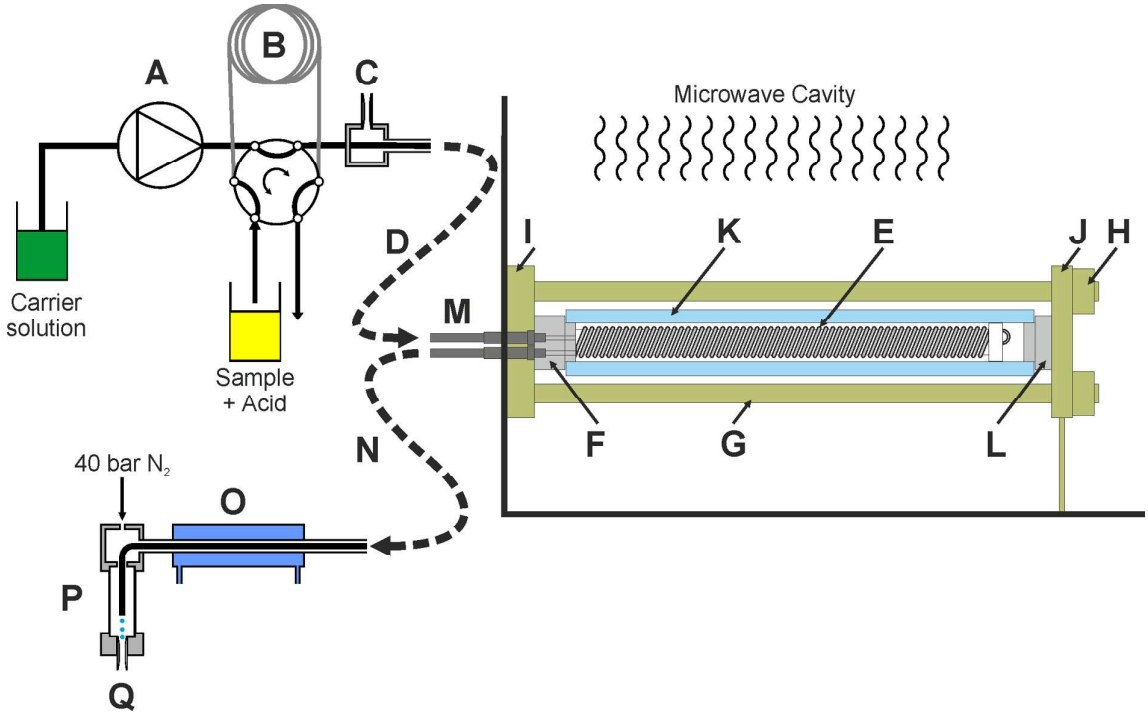

Fig 1. Schematic of the pressure reactor for microwave-assisted flow digestion; A: high pressure pump; B: sample loop and 6-port high pressure valve; C: N_2 exit port of the pressure equilibrium system; D and N: straight stainless steel tubes – dashed line indicates the connection to the high pressure reactor; E: coil former with PFA digestion tube wound around; F and L: PEEK end caps; G: PEEK rods; H: tightening nuts; used to press the base plates onto the end caps; thereby an autoclave is formed inside the glass tube; I and J: PEEK base plates; K: thick walled, precision pulled glass tube (340 mm length, 28 mm inner diameter); M: stainless steel connection tubes; O: cooling unit; P: N_2 inlet of the pressure equilibrium system and sample outlet; Q: sample restrictor capillary

It is important to note, that the liquid volume heated inside the microwave cavity could be easily doubled by winding a second layer of PFA tube on to the coil former. As the first experiments showed satisfactory digestion efficiency this second layer of tube was not deemed necessary.

The maximum sample particle diameter tolerated by this flow digestion system is about half of the inner diameter of the digestion tube. However, depending on the type of sample, gravitational segregation of large particles and swelling or agglomeration during the dissolution reaction must be considered, too.

Instrumentation

Analytes and residual carbon content were quantified either using an axially viewed ICP OES (Ciros Vision EOP, Spectro, Kleve, Germany) or an ICP-MS (Elan DRC+, PerkinElmer, Norwalk, CT, USA) as reported previously[14].

The residual acid concentration in the digested samples was determined by manual titration with 0.10 mol L^{-1} NaOH (Roth, Karlsruhe, Germany) using phenolphthalein as indicator.

Closed vessel microwave digestions were performed in standard ceramic supported PFA vessels ("HF" rotor; maximum pressure of 40 bar) using a commercial microwave digestion system (Multiwave 3000, Anton Paar GmbH, Austria). About 0.25 g solid sample were mixed with the concentrated acids (HNO_3, or mix of HNO_3 and HCl and/or HF). During the first 10 min the microwave power was ramped to 1400 W and thereafter the sample was digested for additional 20 min at the maximum permissible vessel pressure (40 bar).

Thermal images were recorded with an infrared camera (T650sc, FLIR, Boston, MA, USA) immediately after turning off the microwave radiation.

Reagent and samples

All standards solutions and slurries were prepared using high purity acids (subboiled HNO_3; HCl and HF, Suprapur, Merck, Darmstadt, Germany) and purified water (18 MΩ cm, Barnstead Nanopure, Thermo Fisher Scientific, USA). Calibration solutions were prepared from a 100 mg L^{-1} multi element standard solution (Roth, Germany) in 0.5 mol L^{-1} HNO_3 and 1000 mg L^{-1} sulfur and phosphorus standard solution (SCP Science, Quebec, Canada) for elemental analyses and potassium hydrogen phthalate (PA quality, Merck, Germany) for RCC determination.

Glucose, glycine, nicotinic acid and phenylalanine solutions were prepared from respective solid composts of analytical grade (Merck, Germany). The samples investigated in this work were bovine liver and muscle, shrimp, orange and tomato leaves and spinach. The samples were obtained in local markets in São Carlos, SP, Brazil. Moreover, two certified reference materials (CRM) were used in this work: bovine liver (NIST SRM 1577b) and apple leaves (NIST SRM 1515) both produced by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Sample preparation and flow digestion procedure

The animal tissue samples were minced into cubes, put in glass bottles, frozen with liquid nitrogen and lyophilized (MicroModulyo Freeze Dryer, Thermo Scientific, EUA) for 72 h. The

plant tissue was washed with purified water, dried with towel paper, put in paper sacks and dried in oven with forced air circulation (Model 330, Fanem, São Paulo, SP, Brazil) at 60 ºC for 72 h. Then, the animal and plant tissue were ground using a cryogenic mill (CryoMill, Retsch, Haan, Germany), until a fine powder was obtained. It is important to note, that freeze drying and cryogenic milling are not always needed for routine sample digestion. A homogeneous aqueous slurry can also be prepared by mixing water and sample in a laboratory mixer.

Slurries with 1% m/v of solid were prepared from samples or CRM's in purified water and concentrated acids (HNO_3, or mix of HNO_3 and HCl or HF). If not stated differently, the concentration of HNO_3 was 6 mol L^{-1} in all slurries. A homogenizer (Kinematica Polytron PT 3100, Fisher Scientific, Hampton, NH, USA) was used to further homogenize the slurries of animal tissues and orange leaves, while the other samples were preparing just by adjusting the volume and shaking the sample bottles. Scandium was used as internal standard in all slurries. It is important to note, that usually 50 ml slurry were prepared and homogenized in one batch that was subsequently analyzed by introducing several sub-samples into the flow digestion system.

The slurries were digested by the presented fully automated high pressure flow digestion system using the following procedure: 2 ml sample slurry were embedded between two segments of concentrated nitric acid (2 ml each). Thereby a dilution of the slurries acid by the carrier stream could be avoided. The optimized carrier flow rate used was 5 ml min^{-1} at a microwave power of 500 W. With these conditions up to six samples could be digested per hour.

Characterization of the new pressure reactor

The ratio between the vapor- and the liquid phase was determined for different carrier flow rates (2.5 and 5.0 mL min^{-1}) and microwave powers (400, 500 and 600 W) by the following procedure: the flow digestion system was started with a given set of carrier flow rate and microwave power. After 15 min system equilibration, the microwave power was stopped. Immediately after this step, the flow of the carrier stream leaving the reactor through the gas liquid separator stopped because the vapor phase collapsed. The volume of vapor phase inside the reactor can then be determined by multiplying the carrier flow rate with the time elapsed between turning off the microwave and the reappearance of liquid leaving the digestion tube.

The dispersion of analytes through the flow system was evaluated by injecting 2 mL of 10 mg L^{-1} multi element standard solution in 6 mol L^{-1} HNO_3 into the flow system at 500 W microwave power and flow rates of either 2.5 or 5.0 ml min^{-1}. The liquid leaving the flow

digestion system was measured on-line by ICP OES to record transient analyte signals. To avoid the introduction of nitrogen from the gas stream that pressurizes the flow digestion system into the ICP, a second low pressure gas/liquid separator was used and only liquid phase was pumped by a peristaltic pump to the spray chamber of the ICP-OES.

Optimization of the digestion parameters

The digestion efficiency was evaluated from the RCC of digested samples. The substances selected for this purpose were glucose, glycine, phenylalanine and nicotinic acid which are substances that are considered easy, medium, difficult and hard to digest, respectively. A volume of 2 mL of a slurry of either glucose, glycine, phenylalanine or nicotinic acid with a total carbon content of 10.0, 9.0, 5.0 and 9.0 g L^{-1} in 6 mol L^{-1} HNO_3 were digested at different microwave power levels (400, 500 and 600 W) and flow rates (2.5 and 5.0 mL min^{-1}). Phenylalanine was used in lower concentration, as experiments on the previous flow digestion system resulted in clogging of the restrictor capillary by insoluble reaction products formed during incomplete digestion. The digestions were performed off-line and the resulting solutions were analyzed by ICP OES.

Precision and accuracy evaluation

Samples and CRMs were used to evaluate the precision and accuracy of the proposed system and method. In these experiments, 2 mL of sample slurry were digested in the microwave flow digestion system using the optimized conditions obtained in the foregoing experiments (500 W microwave power and 5.0 mL min^{-1} carrier flow rate). The digestion was performed off-line and the digested solutions were analyzed by ICP-OES to determine element concentration and RCC. The obtained results were compared either with the certified values (CRM's) or with values obtained by closed vessel microwave assisted digestion.

Results and discussion

Characterization of the new pressure reactor

Compared to the previously setup, the new pressure reactor installed inside the microwave cavity can contain larger quantities of liquid (13.5 mL vs 6 mL) inside the PFA tube but also has a larger volume pressurized with 40 bar nitrogen. For safety reasons it was deemed necessary to investigate the surface temperature of all components inside the microwave cavity as uncontrolled heating of polymeric (PEEK) components must be avoided.

From Fig 2 it can be deduced that the thick walled glass tube has the highest surface temperature of all components installed inside the microwave cavity (about 130°C). All PEEK components remained below 60 °C even after prolonged system operation. It is important to note, that only steel, glass, PEEK, PFA, PTFE and FFPM O-rings were used inside the microwave cavity.

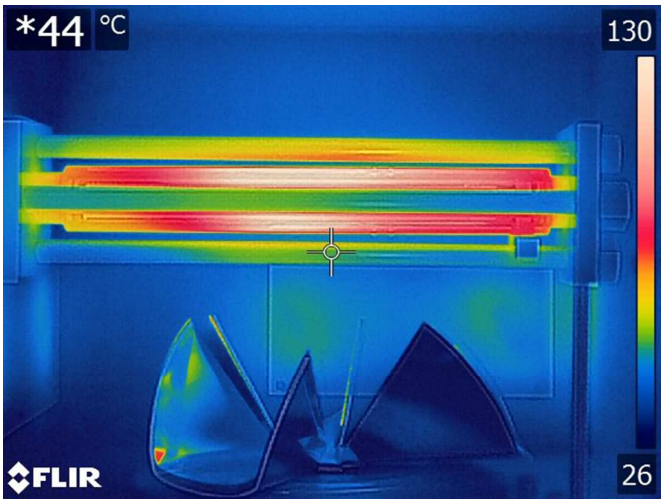

Fig 2. Thermal image of the microwave cavity immediately after turning off the microwave power. Before recording the image the flow digestion system was operated for 15 min at a carrier flow rate of 5 mL min^{-1} and a microwave power level of 500 W. Below the pressure reactor a slowly spinning metallic mode stirrer is arranged for microwave field homogenization.

The ratio between the vapor- and the liquid phase (expressed in %) is an important parameter in flow digestion as it is correlated to the microwave radiation absorption efficiency in the heated zone. A small value indicates little gas phase in the heated zone and consequently low efficient heating. Too vigorous heating on the other hand produces large amounts of vapor phase and results in low digestion efficiency because the digestion solution is quickly ejected from the reactor.

The results shown in Table 1 are surprising. Whereas at a carrier flow rate of 5 mL min^{-1} the ratio between vapor- and liquid phase increases with increasing microwave power level as expected, it decreases for a carrier flow rate of 2.5 mL min^{-1}. Further investigations are necessary to identify the reason for this behavior. With respect to this work it suffices to have clarified that for 400 W or 500 W a carrier flow rate of 2.5 mL min^{-1} or 5.0 mL min^{-1}, respectively are adequate.

Table 1. Ratio between vapor- and liquid phase at different flow rates and microwave power levels, n = 3, NVP: no vapor phase observed

Flow rate (mL min^{-1})	Power (W)	Vapor phase (%)
2.5	300	NVP
	400	15.7 ± 0.5
	500	8.6 ± 0.8
	600	7.8 ± 0.5
5.0	400	NVP
	500	16 ± 1
	600	17.9 ± 0.7

Transient signals recorded for carrier flow rates of 2.5 and 5.0 mL min^{-1}, indicated a broadening of the sample flow pattern by the large volume reactor as the signal duration was 5 and 2 min, respectively. The signal duration was defined as the time elapsed from the first increase of the analyte signal, when the digested sample reached the spray chamber, until the dropping of the signal below the respective limit of quantitation (LOQ). Consequently, the collection time (during this time the sample is collected in a vial at the autosampler) was 9 and 6 min, respectively. The collection time was bigger than the analyte signal duration to ensure that all sample is collected. When including the time needed for sampling the slurry a sample throughput of 4 and 6 samples per hour was attained for slurries that required stirring by the autosampler prior drawing an aliquot into the sample loop. For slurries that didn't tend to segregate over time and that therefore hadn't had to be stirred, a sample throughput of 6 and 9 samples per hour (2.5 and 5.0 mL min^{-1}, respectively) was attained.

Blank digestions (either only 6 mol L^{-1} HNO_3 or 6 mol L^{-1} HNO_3 + 0.7 mol L^{-1} HCl) were analyzed by ICP-MS in order to assess the potential contamination of the digested samples by components of the flow system. Very low concentration of Al (< 1.7 µg L^{-1}), Cr (< 0.4 µg L^{-1}), Cu (< 0.8 µg L^{-1}), Ni (< 0.6 µg L^{-1}) and Zn (< 2.0 µg L^{-1}) were determined (Co, Mn and Mo were below the respective LOQ). However, significant Ti contamination was encountered (up to 150 µg L^{-1}) when the blank solution was mixed with the homogenizer because both, rotor and stator are made from Ti. If Ti should be analyzed in the sample, blenders with ceramic tools must be used.

Optimization of the digestion parameters

Glucose, glycine, nicotinic acid and phenylalanine were used as representative test substances for the determination of the residual carbon content (RCC). Their behavior during digestion is quite different: While glucose reacts violently with nitric acid when heated,

nicotinic acid is very difficult do digest and phenylalanine even forms insoluble intermediate reaction products that require higher temperature to be solubilized again.

The digestion efficiencies for all representative test substances digested under different conditions are presented in Table 2. In general, the digestion efficiencies decreased as follows: glucose > glycine > phenylalanine > nicotinic acid. Nicotinic acid and phenylalanine are substances difficult to digest, because of the aromatic ring in their structures, that requires harsh digestion conditions to be destroyed[18, 19].

Table 2. Effect of the carrier flow rate and microwave power on the digestion efficiency of glucose (Glu), glycine (Gly), phenylalanine (Phe) and nicotinic acid (Nic Ac), mean ± standard deviation, n = 5

Flow rate (mL min^{-1})	Power (W)	Residual Carbon Content (%)			
		Glu	Gly	Phe	Nic Ac
2.5	400	< 1	18.5 ± 0.6	78.6 ± 0.4	94.4 ± 0.5
	500	1.1 ± 0.3	29 ± 1	72.7 ± 0.9	94 ± 1
	600	1.6 ± 0.2	27 ± 1	71.8 ± 0.5	94 ± 2
5.0	400	3.6 ± 0.6	94.2 ± 0.8	76.3 ± 0.7	95 ± 2
	500	2.3 ± 0.5	37 ± 3	77.9 ± 0.7	96 ± 1
	600	1.9 ± 0.5	24 ± 1	80 ± 1	97.2 ± 0.6

At low carrier flow rate (2.5 mL min^{-1}) the RCC rose with increasing microwave power when glucose or glycine were digested. The reason for this behavior is the increased speed of the digestion reaction with fast formation of vapor and gaseous reaction products inside the digestion tube. This exothermic reaction ejected the digestion solution rapidly from the heated zone, reducing the effective digestion time. This fast ejection of gas and liquid was also observed at the end of the digestion tube inside the glass tube P (Fig.1). The behavior of phenylalanine was opposite, as the speed of the digestion reaction is rather low. Therefore, the slight improvement of the RCC can be attributed to an increased temperature at 600 W power.

At a carrier flow rate of 5 mL min^{-1} the RCC decreased with increasing microwave power as the digestion solution was not ejected as fast as at 2.5 mL flow rate. This effect was also clearly observed at the end of the digestion tube. For phenylalanine and nicotinic acid the effect of increasing microwave power was minimal.

Compared to the previously proposed flow digestion system[14] the digestion efficiency was greatly improved: for a 25 g L^{-1} solution of glucose the RCC was reduced from about 24 to < 2 %. A similar improvement could be reached for 28 g L^{-1} glycine (RCC of > 90 % using the previous system compared to – depending on the digestion conditions - about 20 – 40 % in this setup).

Based on the RCC results of the representative test substances, a microwave power of 500 W and a carrier flow rate of 5 mL min^{-1} was selected as compromise conditions for samples of various degrees of reactivity.

The residual acidity of digested solutions of glucose and phenylalanine was 0.85 ± 0.02 mol L^{-1}, without any significant differences among these substances, indicating large excess of nitric acid during the digestion.

Precision and accuracy evaluation

Two CRM's were used to assess the accuracy and precision of the flow digestion procedure. In general, the results listed in Table 3 for HNO_3 and acid mixtures are in good agreement with the certified values. For Fe significantly lower values were obtained when only HNO_3 was used for digestion. When adding HCl to the acid mixture, the Fe-result was not statistically different from the certified value for bovine liver (SRM 1577b). However, for plant material (SRM 1515; apple leaves) the addition of HCl had no beneficial effect. It is well known, that the acid mixture used can affect the results. This behavior can also be observed in closed vessel batch-mode digestion and is not limited to flow digestion.

Also the sulfur concentration determined in bovine liver (SRM 1577b) was lower than the certified value.

Table 3. High pressure flow digestion of CRMs; mean value ± standard uncertainty, n = 5, 95% confidence level, all acid concentration in v/v, ND: not determined, analyte quantification by ICP-OES

Sample	Element	Certified Values	Flow Digestion System		
			HNO_3 6 mol L^{-1}	HNO_3 6 mol L^{-1} + HF 0.6 mol L^{-1}	HNO_3 6 mol L^{-1} + HCl 0.4 mol L^{-1}
Bovine Liver SRM 1577b	Ca (mg/kg)	116 ± 4	120 ± 30	-	108 ± 3
	Cu (mg/kg)	160 ± 8	160 ± 8	-	160 ± 5
	Fe (mg/kg)	184 ± 15	168 ± 5	-	175 ± 5
	Mg (mg/kg)	601 ± 28	590 ± 30	-	580 ± 20
	Mn (mg/kg)	10.5 ± 1.7	8 ± 1	-	7.5 ± 0.2
	P (%)	1.10 ± 0.03	1.05 ± 0.03	-	1.08 ± 0.03
	S (%)	0.785 ± 0.006	0.59 ± 0.08	-	0.67 ± 0.02
	Zn (mg/kg)	127 ± 16	120 ± 5	-	121 ± 4
Apple Leaves SRM 1515	Al (mg/kg)	286 ± 9	250 ± 10	253 ± 10	242 ± 7
	Ba (mg/kg)	49 ± 2	46 ± 2	46 ± 1	51 ± 2
	Ca (%)	1.526 ± 0.015	1.49 ± 0.04	1.56 ± 0.05	1.58 ± 0.05
	Cu (mg/kg)	5.64 ± 0.24	ND	5.0 ± 0.1	5.0 ± 0.2
	Fe (mg/kg)	83 ± 5	67 ± 2	67± 2	64 ± 3
	Mg (%)	0.271 ± 0.008	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01
	Mn (mg/kg)	54 ± 3	47 ± 1	50 ± 1	50 ± 2
	P (%)	0.159 ± 0.011	0.141 ± 0.004	0.14 ± 0.04	0.140 ± 0.004
	Sr (mg/kg)	25 ± 2	22 ± 1	22 ± 1	23 ± 1
	Zn (mg/kg)	12.5 ± 0.3	12.5 ± 0.4	10.3 ± 0.3	12.2 ± 1

In Tables 4 and 5 element concentrations determined in different animal and plant samples are presented. These materials were digested both, by high pressure flow digestion and in a commercial closed vessel batch-mode digestion system, using different acid mixtures. In general, the values obtained by these two digestion approaches were in good agreement.

For the animal tissue samples, it was important to use HCl for the preparation of the slurries because they visually appeared more homogeneous and stable than when only HNO_3 was used. Moreover, the agreement between flow digestion and closed vessel batch digestion was improved. The dispersing effect of HCl was not observed for plant samples. The RCC of the slurries digested with addition of HCl was the lowest among all digestion acid mixtures evaluated. The obtained values were 33 ± 1, 24 ± 2 and 33 ± 3 mg L^{-1} for bovine liver, bovine muscle and shrimp, respectively, while they were 61 ± 3, 54 ± 2 and 56 ± 3 mg L^{-1} for the same sample sequence digested only with HNO_3 in the flow digestion system. For plant tissues, the RCC was between 5 ± 3 and 18 ± 1 mg L^{-1} independently on the sample digested or the acid mixture used.

Table 4: Comparison of flow- and closed vessel batch digestion of bovine liver, bovine muscle and shrimp in different acid solutions, mean value ± standard uncertainty, n = 5, 95% confidence level, ND: not determined, a: HNO_3 6 mol L^{-1}, b: HNO_3 6 mol L^{-1} and HCl 0.4 mol L^{-1}, c: HNO_3 10 mol L^{-1} and HCl 1.6 mol L^{-1}, analyte quantification by ICP-OES

Sample	Element	Flow Digestion System		Clossed Vessel Digestion
		HNO_3[a]	HNO_3 + HCl[b]	HNO_3+ HCl[c]
Bovine Liver	Ca (mg/kg)	150 ± 30	137 ± 4	146 ± 5
	Cu (mg/kg)	264 ± 8	259 ± 8	253 ± 8
	Fe (mg/kg)	194 ± 6	171 ± 5	163 ± 5
	Mg (mg/kg)	570 ± 20	580 ± 20	550 ± 20
	Mn (mg/kg)	7.5 ± 0.2	7 ± 1	7.1 ± 0.2
	P (%)	1.18 ± 0.04	1.18 ± 0.04	1.25 ± 0.04
	S (%)	0.71 ± 0.02	0.73 ± 0.02	0.70 ± 0.02
	Zn (mg/kg)	113 ± 3	113 ± 3	109 ± 3
Bovine Muscle	Ca (mg/kg)	120 ± 30	120 ± 20	131 ± 7
	Fe (mg/kg)	55 ± 2	60 ± 2	60 ± 2
	Mg (mg/kg)	870 ± 30	860 ± 30	820 ± 30
	P (%)	0.73 ± 0.02	0.72 ± 0.02	0.77 ± 0.02
	S (%)	0.73 ± 0.02	0.74 ± 0.02	0.75 ± 0.02
	Zn (mg/kg)	149 ± 4	145 ± 4	141 ± 4
Shrimp	Al (mg/kg)	24 ± 1	33 ± 3	39 ± 1
	Ca (%)	0.42 ± 0.01	0.43 ± 0.02	0.38 ± 0.01
	Cu (mg/kg)	ND	7 ± 2	5.7 ± 0.2
	Fe (mg/kg)	47 ± 1	30 ± 1	31 ± 1
	Mg (mg/kg)	2020 ± 60	2040 ± 60	1900 ± 60
	Mn (mg/kg)	7.5 ± 0.2	7.5 ± 0.2	7.7 ± 0.2
	P (%)	1.39 ± 0.04	1.40 ± 0.04	1.44 ± 0.04
	S (%)	1.09 ± 0.03	1.12 ± 0.03	1.09 ± 0.03
	Sr (mg/kg)	60.0 ± 0.2	63 ± 3	57 ± 2
	Zn (mg/kg)	49 ± 1	51 ± 2	48 ± 1

Table 5: Comparison of flow- and closed vessel batch digestion of spinach, tomato and orange leaves in different acid solutions, mean value ± standard uncertainty, n = 5, 95% confidence level, ND: not determined, a: HNO_3 6 mol L^{-1}, b: HNO_3 6 mol L^{-1} and HF 0.6 mol L^{-1}: HNO_3 6 mol L^{-1} and HCl 0.4 mol L^{-1}, d: HNO_3 10 mol L^{-1}, HCl 1.6 mol L^{-1} and HF 1 mol L^{-1}, analyte quantification by ICP-OES

Sample	Element	Flow Digestion System			Closed Vessel Digestion
		HNO_3[a]	HNO_3+ HF[b]	HNO_3+ HCl[c]	HNO_3+ HCl+ HF[d]
Spinach	Al (mg/kg)	53 ± 5	34 ± 5	53 ± 5	41 ± 3
	Ba (mg/kg)	2.5 ± 0.1	2.5 ± 0.1	5.0 ± 0.1	3.2 ± 0.1
	Ca (%)	1.66 ± 0.05	1.73 ± 0.05	1.73 ± 0.05	1.6 ± 0.1
	Cu (mg/kg)	ND	6 ± 2	7 ± 2	7.6 ± 0.2
	Fe (mg/kg)	100 ± 3	101 ± 3	95 ± 3	92 ± 7
	Mg (%)	1.49 ± 0.04	1.51 ± 0.04	1.53 ± 0.05	1.2 ± 0.1
	Mn (mg/kg)	136 ± 4	135 ± 4	134 ± 4	129 ± 4
	P (%)	0.55 ± 0.02	0.55 ± 0.02	0.54 ± 0.02	0.55 ± 0.03
	S (%)	0.46 ± 0.04	0.48 ± 0.03	0.47 ± 0.03	0.52 ± 0.02
	Sr (mg/kg)	112 ± 3	108 ± 3	109 ± 3	105 ± 3
	Zn (mg/kg)	49 ± 3	47 ± 3	48 ± 4	51 ± 2
Tomato Leaves	Al (mg/kg)	88 ± 7	71 ± 7	79 ± 8	98 ± 3
	Ba (mg/kg)	79 ± 2	77 ± 2	81 ± 2	72 ± 2
	Ca (%)	3.8 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	3.8 ± 0.3
	Cu (mg/kg)	ND	10 ± 2	12.3 ± 0.4	11.7 ± 0.4
	Fe (mg/kg)	205 ± 6	211 ± 6	196 ± 6	206 ± 13
	Mg (%)	0.49 ± 0.01	0.49 ± 0.01	0.50 ± 0.02	0.45 ± 0.03
	Mn (mg/kg)	73 ± 2	75 ± 2	75 ± 2	70 ± 2
	P (%)	0.29 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.29 ± 0.01
	S (%)	1.00 ± 0.07	1.03 ± 0.08	1.00 ± 0.08	0.97 ± 0.03
	Sr (mg/kg)	188 ± 6	180 ± 5	185 ± 6	173 ± 7
	Zn (mg/kg)	16 ± 1	15 ± 1	16 ± 1	16.9 ± 0.5
Orange Leaves	Al (mg/kg)	29 ± 4	22 ± 8	30 ± 3	27.6 ± 0.8
	Ba (mg/kg)	26 ± 1	26 ± 2	25 ± 2	23.4 ± 0.7
	Ca (%)	1.75 ± 0.05	1.9 ± 0.1	1.83 ± 0.05	1.77 ± 0.08
	Fe (mg/kg)	70 ± 5	80 ± 5	62 ± 2	67 ± 2
	Mg (%)	0. 30 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.29 ± 0.01
	Mn (mg/kg)	15.0 ± 0.4	17 ± 1	15.0 ± 0.5	15.3 ± 0.5
	P (%)	0.19 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
	S (%)	0.21 ± 0.02	0.23 ± 0.01	0.23 ± 0.01	0.24 ± 0.02
	Sr (mg/kg)	60 ± 2	63 ± 3	60 ± 2	58 ± 2
	Zn (mg/kg)	14 ± 1	13 ± 2	15 ± 2	15 ± 1

Conclusions

The increase of the reactor volume improved the digestion efficiency (i.e. lower RCC for organic samples) significantly when compared with the previous high pressure flow digestion system[14]. The setup was able to perform digestion of slurries without clogging problems even when introducing coarse or fibrous samples prepared in the laboratory from plants or animal tissues. Results obtained by high pressure flow digestion were in good agreement with the certified values (CRMs) or the values obtained by closed vessel microwave-assisted digestion. The system was found to operate highly reliable and fully automated over a prolonged period of time. Although highly corrosive acids like HCl or HF were used in the digestion acid mixture, no elevated blanks or system corrosion was encountered.

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