

Using high-pressure teflon bomb digestion in phosphorus determination of aquatic animals

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Abstract – High pressure and temperature were combined with the convenient mixture of acids to perform an effective digestion of samples from aquatic animals for phosphorus (P) analysis. Our results indicate that digestion in high-pressure bombs combined with concentrated acidic media can be a convenient way to yield all P stored in samples, and to produce accurate recoveries. Phosphorus recovery of a certified standard material ($94\% \pm 15$) and digestions of aquatic animal tissues confirmed the relevance of bomb decomposition, as well as its reliability compared to ashing and microwave oven digestion methods. Moreover, the applied digestion technique is easily manageable even in samples consisting of a few individuals. This method is relatively rapid, saves money and time, and is able to recover P from the most persistent components (*e.g.* bones, scales, fat) of aquatic animal tissues.

Key words: High-pressure digestion / phosphorus / fish / zooplankton / benthos

Introduction

Increased knowledge about the nutrient content of aquatic animals is important for understanding their role in cycling, transport and storage of nutrients. Major nutrients, such as carbon and nitrogen can be measured in dried-homogenized samples with good accuracy and precision in a relatively short time using carbon-hydrogen-nitrogen analyzers (Tanner *et al.*, 1999; Sterner and George, 2000; Pilati and Vanni, 2007; Le Loc'h *et al.*, 2008). In contrast, phosphorus (P) analysis is more time-consuming and requires that animal tissues are digested before colorimetric measurement. The traditional standard method for determination of P is performed by oxidizing sample total phosphorus content to dissolved orthophosphate by digestion and colorimetric determination of liberated dissolved orthophosphate ions (Colina *et al.*, 1996). The principle of the colorimetric measurement is the formation of 1,2-molybdophosphoric acid which is reduced to the phosphomolybdenum blue complex in the presence of reducing agent. The intensity of the blue colour is proportional to the amount of orthophosphate ions incorporated into the complex and hence, the reacted orthophosphate can be determined (Jarvie *et al.*, 2002). However, the molar ratio of $[H^+]$ and $[MoO_4^{2-}]$

may influence the outcome of colorimetric measurements and induce “non-phosphate sensitized MoO_4^{2-} ion reduction” (Pai *et al.*, 1990).

For digestion, a combination of sulphuric acid and nitric acid is proven as an effective tool (Brown *et al.*, 1973; Sadiq and Zaidi, 1983; Colina *et al.*, 1996), whilst higher pressure of a closed and heated system has a strong positive influence on the digestion of persistent materials (Schnitzer *et al.*, 1995). Scientists use various digestion protocols for the same sample types and the results may not be comparable among procedures. The diversity of methods used to digest tissues from aquatic animals illustrates well this variety. For instance, Higgins *et al.* (2006) determined P content by ashing samples in a muffle furnace, followed by hydrochloric acid digestion. Sterner and George (2000) used ashing as well, but dissolved the ash in diluted (0.3 N) nitric acid. Hendrixson *et al.* (2007) used hot 10 N sulphuric acid, and Sereda *et al.* (2008) used 70% nitric acid for digestion of fish in the same procedure.

The so-called wet digestion method, applied when ashing is not included in the process, is also used widely. Frost *et al.* (2003) digested aquatic invertebrate samples by autoclaving with a potassium-persulfate digestion reagent. Tanner *et al.* (1999, 2000) and Reis *et al.* (2008) used concentrated acids and microwave oven treatment to digest dried biological samples. These procedures are

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based on the joint capacity of heat, pressure and oxidizing media to disassemble sample materials.

The same principle is relevant for high-pressure bomb digestion, which has been successfully applied in sediment P digestion (Colina *et al.*, 1996). High pressure bombs are commonly used tools in the wet digestion of biological samples in heavy metal determination (Farkas *et al.*, 2002, 2003; Ringmann *et al.*, 2002). However, we have not found any references regarding the use of this method in P analysis of aquatic animal samples.

To confirm the validity of teflon bomb digestion technique in P content analyses, we tested the digestibility and recovery rate of a certified standard material and the results were compared with the efficiency of the commonly used “ashing in muffle furnace” and “microwave oven digestion” methods. In addition, we have digested fish, zoobenthos, and zooplankton samples and compared the obtained P contents to those available in the literature on the same taxonomic groups being digested by other methods.

Materials and methods

Four different samples were analyzed in our study: pork muscle standard (NCS ZC 81001, China National Analyst Centre for Iron and Steel) with certified P content ($0.813\% \pm 0.031$), homogenized whole body of a cyprinid fish, Prussian carp (*Carassius auratus* L., TL = 44 cm, W = 1660 g), macro-zoobenthos (chironomid larvae) and cladoceran zooplankton. All samples were dried to a constant weight at 65 °C and milled to a fine powder by an electric grinder. To ensure homogenization, samples were passed twice through the grinder. Before weighing, samples were ground again with a mortar and pestle. The pork muscle standard was homogenized to a fine powder by the producer company. Teflon vessels and glassware used in measurements were washed with concentrated potassium dichromate ($K_2Cr_2O_7$) followed by a de-ionized water rinse. All chemicals were analytical reagent or puriss grade.

For sample digestion, our own, so-called high-pressure teflon bombs were used, which were manufactured at the University of Pannonia, Hungary. The construction is similar to the General Purpose Acid Digestion Bombs, distributed by Parr Instrument Company, USA. A teflon bomb consists of an aluminium holder, a teflon and aluminium cap with a screw-topped closing system and a removable teflon vessel (100 mL volume, $d = 4.2$ cm, $l = 7$ cm) inside (Fig. 1).

Dried samples (150–300 mg) were weighed to place in the teflon vessel, using a Sartorius BA 210S analytical balance, then 10 mL of acid mixture (96 m% sulphuric acid:65 m% nitric acid; 3:2) were added. Vessels were capped, placed into their aluminium holder and closed tightly with the screw system. In addition to bombs with samples, each digestion run contained a blank run as a control (to detect the background with 10 mL of acid mixture and free of biological material). Bombs were

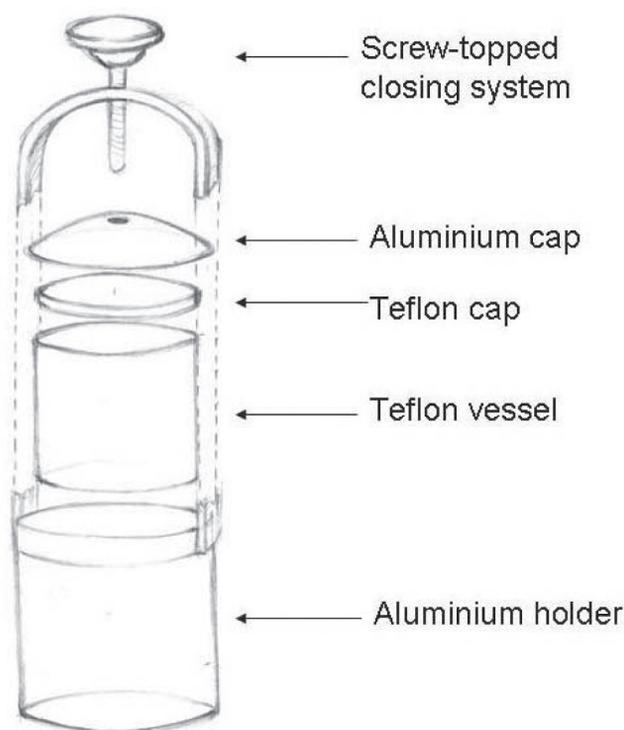


Fig. 1. Sketch of the high-pressure teflon bomb used for digestion of aquatic animal samples.

heated to 100 °C on an electric oven plate for 4 h. Pressure in the bombs was not defined. Following cooling, vessels were opened in a fume hood and 0.5 mL digested sub samples were pipetted into scintillation vials with three replicates from each bomb. Subsequently, 9.5 mL of de-ionized water was added to the sub samples, as the first twenty-fold dilution step to complete the acid hydrolysis. Samples were stored in a dark place at room temperature overnight, then 0.5 mL was pipetted from the diluted solution and 9.5 mL de-ionized water was added. Final dilution of samples was four hundred-fold and after this step, they were ready for P-content determination.

Phosphorus content was measured spectrophotometrically at 880 nm (Shimadzu UV 160-A spectrophotometer) using the conventional ammonium molybdate method (Strickland and Parsons, 1972). Relatively low (< 60) molar ratios between $[H^+]:[MoO_4^{2-}]$ might result in self-reduction of the MoO_4^{2-} ion to form a molybdenum blue colour independent of the P concentration (Going and Eisenreich, 1974; Pai *et al.*, 1990). As our digesting reagents were concentrated acids and pH was relatively low in samples, we relied on the non-phosphate sensitized MoO_4^{2-} ion reduction. Thus, mean absorbance measured in the blanks was subtracted from the absorbance of the samples. Hereby, we could eliminate the background absorbance produced by the digestion reagents. To get precise data from each subsample, we calculated the mean and standard deviation of three sub-replicates.

Table 1. Comparisons of P-contents of a cyprinid fish (Prussian carp), benthic invertebrates (chironomids) and cladoceran plankton measured following high-pressure bomb digestion in our study and that of other digesting procedures.

	High-pressure bomb digestion (this study) × mean $1 \pm$ SD (dry w^{-1} , %) (min–max; <i>n</i>)	Other digesting methods × mean $1 \pm$ SD (dry w^{-1} , %) or range
Cyprinid fish	2.70 ± 0.32 (2.30–3.30; 7)	2.71 ± 0.26 † (Penczak and Tátrai, 1985) 0.80–3.20* (Sterner and George, 2000) 2.40–3.60* (Hendrixson <i>et al.</i> , 2007) 2.10–4.20* (Sereda <i>et al.</i> , 2008)
Benthic invertebrates-chironomids	1.04 ± 0.10 (0.90–1.15; 7)	0.85 ± 0.17 ** (Frost <i>et al.</i> , 2003)
Cladoceran plankton	1.30 ± 0.19 (0.99–1.54; 7)	1.4 ± 0.2 ** (Andersen and Hessen, 1991) 1.2 – 1.4 ** (Walve and Larsson, 1999)

Digestion methods: * ashing and acid hydrolysis; ** potassium-persulfate digestion; † no reference for applied digestion.

Results

High-pressure digestion carried out in our teflon bombs in concentrated acids resulted in high recovery rate ($\bar{x} \pm 1$ SD; $94 \pm 15\%$, range: 79–115%, $n = 9$) for pork muscle standard. Blank values, measured in the bombs with digesting chemicals and free of biological material resulted in a relatively high absorbance ($92 \pm 2.9 \mu\text{g P.L}^{-1}$). This background was then used for correction of our measured P-contents.

P contents of cyprinid fish, benthic invertebrates (chironomids) and cladoceran plankton digested with the teflon bomb method are presented in Table 1. Our measured values proved to be comparable with those digested either by ashing or potassium-persulfate.

Discussion

Our results indicate that digestion in our high-pressure teflon bomb combined with concentrated acidic media can be a convenient way to yield total P content of various sample types and produce accurate recoveries. Mean recovery rate for standard material is similar to that of obtained following microwave oven digestion (90–95%) and is by 14% higher than the recovery rate (80%) found using ashing method combined with nitric acid hydrolysis (Tanner *et al.*, 1999). Mean recovery from standard material was close to the certified value following correction with the blank value, which supports the theory of P-independent MoO_4^{2-} ion reduction (Going and Eisenreich, 1974; Pai *et al.*, 1990). We found that acid background elimination in the evaluation of the results can be a reliable way to calculate P content after digestion and colorimetric measurements.

Strong oxidation capacity of concentrated acids combined with high pressure and temperature resulted in digestion of the most recalcitrant materials such as fat, bony particles, scales or chitin. Moreover, there were no particulate sample remains in our teflon vessels after digestion. Ashing, a widespread method prior to colorimetric measurements (Sterner and George, 2000; Hendrixson *et al.*, 2007; Sereda *et al.*, 2008) decomposes complex and recalcitrant materials and solves the problem of absolute homogenization. Procedure of ashing may

reduce sample mass by an order of magnitude, however, in case of small sample size (*e.g.* 1–2 individuals of macro-invertebrates or few zooplankton) it is not precisely applicable as ash is usually reweighed for P analysis. Sample digestion in our high-pressure teflon bomb is manageable even in case of samples consisting of few individuals only. Moreover, high-pressure bomb digestion is relatively rapid and the expenses of the analysis are low, which ensures a widespread application of this method.

The variety in different P digestion methods clearly reveals that researchers are not consistent in this issue (Tanner *et al.*, 1999; Sterner and George, 2000; Sereda *et al.*, 2008), thus we support international standardization to achieve results comparable among laboratories. We have broadened the application of high-pressure bomb digestion to give an alternative tool and reference for limnologists doing measurements on P stoichiometry of any kind of aquatic animals.

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