

## Microwave digestion method for phosphorus determination of fish tissue

**Abstract**—A simple time-saving method is described for the digestion of fish tissue for spectrophotometric determination of phosphorus. Microwave digestion of fish tissue was compared to the commonly used method of ashing tissue in a muffle furnace. Microwave digestion produced significantly higher phosphorus recoveries and took only half the time to run the same number of samples compared to ashing.

Increased knowledge about the nutrient content of biological organisms is essential for a thorough understanding of ecological stoichiometry (Sterner 1995; Elser et al. 1996) and nutrient transport (Deegan 1993; Vanni 1995) in and among ecosystems. Of particular importance are carbon, nitrogen, and phosphorus, which are essential for both “protoplasmic life” and “mechanical structures” (Reiners 1986). Carbon and nitrogen can be measured in dried fish tissue with good accuracy and precision in a relatively short time using a carbon–hydrogen–nitrogen analyzer. However, spectrophotometric phosphorus analysis requires that fish tissue be digested or ashed, and previously developed methods are either hazardous or tedious and time-consuming. Ashing, a commonly used but time-consuming procedure originally developed for plant tissue (Jackson 1958), combusts samples in a muffle furnace. Perchloric acid/nitric acid has also been widely used to digest tissues (Becker et al. 1992), but it is potentially explosive and requires specially designed hoods (American Public Health Association 1995). This paper describes a third method utilizing microwave acid digestion and compares the results obtained with this method to those obtained using the ashing method. Microwave digestion has been used for other biological samples with good results (Nadkarni 1984; White and Douthit 1985; White 1988; Collins et al. 1996). However, there are currently no published methods in the readily available literature that are not overly time-consuming or that can be completed without specialized perchloric acid fume hoods.

Two types of samples were analyzed for this study: a field-collected 290-g northern pike *Esox lucius* and standardized dried oyster tissue (0.623% P) obtained from the National Institute of Standards and Technology (NIST). The northern pike was collected in August 1995 from Bark Bay Slough, which is located on the Wisconsin shore of Lake Superior, 10 mi. east of Port Wing. The northern pike sample was frozen for 8 months and processed using a large food grinder (Hobart, model 4532). To ensure homogenization, all pike tissue was passed through the grinder three times. Following processing, tissue was dried in a convection oven for 24 h at 60°C, ground with a mortar and pestle, placed in a glass jar, tightly capped, and stored in a freezer. The oyster tissue was ground to a homogeneous mixture by NIST.

All glassware was prerinse with 10% HCl followed by deionized water. All reagents were analytical reagent grade. Both the  $\text{KH}_2\text{PO}_4$  used as a phosphorus standard and the

oyster tissue were certified reference materials. Two hundred fifty milligrams of dried fish or oyster tissue was weighed directly in the microwave digestion vessel (120-ml Teflon cylinder 4-cm I.D.  $\times$  11 cm long with a ventable screw cap). Ten milliliters of concentrated  $\text{HNO}_3$  (70% by weight) was added, and each vessel was capped, torqued to specifications in the CEM Corporation capping station, and placed in a CEM MDS 81D microwave oven. This 650-W microwave oven was operated 5 min at 30% power, 5 min at 40% power, 5 min at 50% power, 5 min at 0% power, and 30 min at 65% power. Some fish and oyster samples were treated additionally with 30%  $\text{H}_2\text{O}_2$  for 30 min at 65% power. After 1 h of cooling, vessels were removed from the microwave, vented, and opened in a fume hood. One drop of phenolphthalein solution was added to each sample prior to neutralizing with 10 N NaOH until a pink to orange color was obtained. Neutralization was done slowly (dropwise initially) to avoid sample eruptions out of the vessel. Following neutralization, 50%  $\text{HNO}_3$  was added dropwise until samples turned light yellow to colorless. Samples were quantitatively transferred from microwave vessels to 50-ml volumetric flasks for phosphorus analysis.

Two muffle furnace ashing methods were used to prepare 250 mg of dried fish or oyster tissue (Davis and Boyd 1978; Analytical Methods Committee 1979). Phosphorus analysis from the microwave and muffle furnace digestion was accomplished using the vanadomolybdophosphoric acid method with a Perkin-Elmer model LAMBDA 2 UV/VIS spectrophotometer set at a wavelength of 420 nm (Analytical Methods Committee 1979).

Differences in results from the various digestion methods were compared using a one-way analysis of variance (ANOVA).

Phosphorus values for our standard fish tissue (northern pike) ashed in a muffle furnace and digested in  $\text{H}_2\text{SO}_4$  (Analytical Methods Committee 1979) were significantly lower than those obtained after microwave digestion with either  $\text{HNO}_3$  ( $P = 0.034$ ) or  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  ( $P = 0.003$ ) (Table 1). Phosphorus recoveries from ashing followed by digestion in  $\text{HNO}_3$  (Davis and Boyd 1978) were also significantly lower than those obtained after microwave digestion with either  $\text{HNO}_3$  or  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  ( $P < 0.001$ ).

Oyster tissue phosphorus recovery did not follow the same trends across digestion methods as the northern pike tissue. Microwave digestion in  $\text{HNO}_3$  plus 30%  $\text{H}_2\text{O}_2$  yielded the highest recovery (95%) of phosphorus in oyster tissue, followed by ashing with  $\text{H}_2\text{SO}_4$  digestion (93%). White and Douthit (1985) also recovered 95% phosphorus in oyster tissue using a microwave oven and nitric acid/hydrogen peroxide digestion. Microwave digestion with  $\text{HNO}_3$  alone resulted in a phosphorus recovery of 90%, while ashing with  $\text{HNO}_3$  digestion yielded the poorest recovery (80%). Microwave digesting with  $\text{HNO}_3$  and with  $\text{HNO}_3$  plus 30%  $\text{H}_2\text{O}_2$  yielded significantly higher percent phosphorus recoveries

Table 1. Comparison of mean phosphorus tissue values (% dry weight) using microwave and muffle furnace digestion of northern pike and oyster tissue.

	Microwave			
	Microwave HNO <sub>3</sub>	HNO <sub>3</sub> and H <sub>2</sub> O <sub>2</sub>	Muffle H <sub>2</sub> SO <sub>4</sub>	Muffle HNO <sub>3</sub>
Northern pike				
All tissues $\bar{x}$	2.05*	2.07*	1.91†	1.47‡
Range	1.79–2.27	1.90–2.28	1.77–2.17	1.11–1.85
SD	0.15	0.11	0.11	0.21
C.V.	7.4	5.3	5.8	14.3
<i>n</i>	10	11	11	11
Oyster				
All tissues $\bar{x}$	0.56†	0.59*	0.58†	0.50‡
Range	0.53–0.59	0.57–0.63	0.57–0.59	0.48–0.55
SD	0.021	0.017	0.0068	0.021
C.V.	3.8	2.9	1.2	4.2
<i>n</i>	8	11	10	11
% recovery§	90	95	93	80

\*†‡ Mean phosphorus tissue values with dissimilar coefficients are significantly different ( $P < 0.05$ ).

§ Based on NIST value of 0.623% phosphorus.

( $P < 0.001$ ) than muffle furnace ashing and HNO<sub>3</sub> digestion. Microwave digestion with HNO<sub>3</sub> plus 30% H<sub>2</sub>O<sub>2</sub> produced significantly higher phosphorus recoveries ( $P = 0.008$ ) than microwave digestion with only HNO<sub>3</sub>. Although microwave digestion of fish tissue provides nearly identical results with HNO<sub>3</sub> or HNO<sub>3</sub> plus 30% H<sub>2</sub>O<sub>2</sub>, phosphorus recovery can be improved in oyster tissue using microwave digestion with HNO<sub>3</sub> plus 30% H<sub>2</sub>O<sub>2</sub> or ashing followed by digestion with H<sub>2</sub>SO<sub>4</sub>. Apparently, nitric acid is not a strong enough oxidizing agent to digest the oilier oyster tissue, but it is adequate for digestion of northern pike tissue. Sulfuric acid cannot be used in the closed microwave vessels because it will damage the vessels at the higher pressures and resulting higher boiling temperatures. The closed system of the microwave digestion vessels with resulting minimal losses to volatilization may partially explain the higher phosphorus recoveries of this method. The Teflon vessels vent only when the inside pressure exceeds 830 kPa. The very poor recoveries of the ashing method of Davis and Boyd (1978) may be due to the lack of an ashing aid (magnesium acetate) (Jackson 1958), lower muffle furnace temperature and shorter time, and because nitric acid is not as strong an oxidation agent as sulfuric acid.

The microwave method of digesting fish tissue resulted in significantly higher phosphorus recoveries and only required half the time of the muffle furnace methods. Microwave digestion is a reasonably safe method that produces accurate recoveries with good precision.

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