HORTSCIENCE 26(12):1496-1497. 1991,

Microwave Digestion of Plant Samples for Boron Analysis

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Additional index words. Prunus persica, Lycopersicon esculentum, Citrus sp., foliar analysis, plasma emission spectrometry, plant nutrition, sulfur nutrition

Abstract. **The closed vessel microwave procedure developed requires less than 2 hours to produce 12 digested 0.50-g samples ready for B analysis by plasma emission spectrometry. The predigestion procedure uses nitric acid and hydrogen peroxide. The predigested samples are then each microwaved for 2.25 minutes (12 samples, 27 minutes). With this procedure, B concentration levels are statistically the same as the value determined for dry ash-digested materials. Recovery levels of known quantities of B were above 989%. Boron and S concentrations could be obtained from the same plant digest. This method produced results for four samples each of citrus, tomato, and peach leaves with SD of 0.596, 0.824, and 0.350 μg B/g, respectively.**

Deficiencies of B are common in horticultural crops and they significantly affect quality (Mengal and Kirby, 1987). The difference between a sufficient level and a toxic level of B covers a relatively short range (Mengal and Kirby, 1987). In diagnosing-B nutrition problems, accurate B determination must, therefore, be obtained.

The quest for an appropriate plant tissue analysis for B is complicated by its chemistry and the opportunity for contamination. The severity of the problem is reflected in the fact that the National Bureau of Standards (NBS) does not offer a certified value for Bin any plant samples. Wickner (1986) characterizes the problems of B analysis as inaccuracies during sample digestion due to contamination from glassware, loss from high temperatures, or loss due to low pH. Two methods of digesting plant materials for B analysis are generally accepted. The sodium hypobromite digestion is used when analysis is completed by a modified curcumin method (Kowalenki, 1979). When plasma emission spectrometry is used, dry ashing with or without CaO followed by the addition of concentrated HCl is the accepted procedure (Wickner, 1986). Both methods are demanding in attention, time, and materials.

Received for publication 25 Jan. **1991.** The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked ***advertisement solely*** to indicate this fact.

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Recent work on microwave digestion for plant materials offers a potential for a simpler and faster procedure (Finch et al., 1990). This paper addresses closed-vessel microwave digestion followed by plasma emission spectrometry analysis as a tactic for effective B analysis of plant materials. The 16-step procedure and equipment described by Finch et al. (1990) were used.

We used a 600-W microwave oven fitted with a 12-vessel carousel prepared by the manufacturer for plant sample digestion. A 0.50-g sample of ground (1.0-mm screen) plant material is covered in the digestion vessel with 10 ml of HNO_3 and left to react 30 min. Hydrogen peroxide (2.5 ml) is added to the mixture and swirled. When the frothy reaction fills 75% of the digestion vessel, it is capped and the vent tube is fitted. The carousel of capped vessels is placed in the oven at 95% power for 2.25 min for each sample. After the samples cool, 35.5 ml of distilled water is added to each sample and the mixture is analyzed as a 0.5-g sample in a 45.5-ml dilution on the inductively coupled plasma emission spectrometer.

In discussion of digestion procedures, temperatures of 550 to 660C are cited as a cause of B losses due to volatilization (Wickner, 1986). The temperatures in our procedure stayed well below these levels (Table 1). The same study cites the difficulty of using nitric acid as a digestive reagent without reducing B recovery. The loss appears to be limited by the addition of H_2O_2 and by the use of closed vessels in the procedure (Wickner, 1986). The digestion vessel in our procedure is not opened until the material has cooled to room temperature and it uses H_20_2 for predigestion, which may explain why. accuracy remains high despite the use of nitric acid.

Plant materials digested by the microwave method and analyzed for B provide reasonably consistent results that compare well with dry-ash digestions (Table 2). The microwave

Table 1. Temperatures and pressures attained within the closed vessels using microwave digestion for plant materials (Citrus leaves NBS Standard Reference Material no. 1572).

Time (min)	Temp (°C)	Pressures (kPa)
0	28	0
3	93	41
6	113	179
9	138	566
12	147	883
15	146	890
18	148	883
21	150	876
24	152	883
27	154	883

Table 2. Comparison of B contents of citrus leaf standard samples prepared by dry-ash and microwave digestion methods.

Citrus standard (no.)	Dry-ash digestion (μg B/g dry wt)	Microwave (μg B/g dry wt)	Difference from dry ash (%)
1	61.13	59.77	−2.2
2	59.70	58.84	−1.4
3	54.45	57.99	6.6
Mean ± SD	58.43 ± 3.52	58.87 ± 0.89	0.75

Table 3. Boron recovery from samples of distilled water with known quantities of H_3B0_3 or known quantities of certified B standard with $(NH_4)_2B_4O_4 \cdot H_2O$ as the B source.

Sample		Recovery		
Type	No.	Expected (μg B/g dry wt)	Actual (μg B/g dry wt mean ± SD)	%
H_3BO_3	2	130.00	129.67 ± 1.53	99.7
H_3BO_3	3	85.00	84.99 ± 1.33	100.0
Certified Standard	3	40.00	39.31 ± 0.49	98.3
Certified Standard	3	20.00	19.75 ± 1.32	98.8

Table 4. Results of B analysis for leaves digested by the closed-vessel microwave digestion procedure.

Plant	Mean µg B/g dry wt (4 samples ± SD)
Citrus	60.47 ± 0.596
Tomato	46.44 ± 0.824
Peach	27.82 ± 0.350

digestion method produces a range of 6.5% more B to 2.2% less B than does dry-ash digestion for three samples of NBS Standard Reference Material citrus leaf. The difference between the two digestion methods was not significant at the 0.95 confidence level.

Recovery rates were above 98.3% for B concentrations of 20, 40, 85, and 130 µg B/g dry weight (Table 3). The samples containing 85 and 130 µg B/g dry weight, respectively, also contained 1000 and 2000 µg S/g in the form of $(NH_4)_2SO_4$. The S recovery levels matched those reported by Finch et al. (1990). The recovery of B in the samples that included S ranged from 98.3% to 100.0% for two levels of B, indicating that the two elements could be analyzed from one digestion (Finch et al., 1990).

Although comparisons between dry-ashed and microwaved samples of peach [Prunus *persica* (L.) Batsch] and tomato *(Lycopersicon esculentum* Mill.) leaves were not run, separate digestions of the same sample for the two species show consistent B levels, as judged by the SD (Table 4). There is no reason to believe that these and other foliar samples could not be analyzed with the same accuracy as citrus leaves.

The closed-vessel microwave digestion method for B analysis was pursued in this study because standard rapid analysis procedures that were available did not give consistently accurate results for the important nutrient, and the dry ashing and sodium hypobromite digestion procedures were not rapid and simple enough for a production laboratory. We believe that the procedure also would work for multi-element analysis.

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