Need for $Ca(OH)_2$ in Boron Determination in Plant Vegetative Tissue and Oilseeds

Negligible differences in the boron content of the vegetative organs of groundnut (peanut, TMV-2) and leaves of 11 other plants were found in samples ashed at 550° C. with or without the addition of $Ca(OH)_2$. Approximately one third of the boron was lost from oilseeds such as groundnut, gingelly, castor, and Indian mustard when the samples were ignited without added $Ca(OH)_2$. The addition of a base prior to ashing is not necessary for determination of boron in the vegetative tissue of plants, but is advisable in the case of oilseeds.

The boron content of plant material was determined by mixing the material with excess alkali and ashing it at low temperature (McHargue and Calfee, 1932). Later, boron was determined by ashing plant samples at 550° C. (Hatcher and Wilcox, 1950), 500° C. (Higgons, 1951), and 600° C. (MacDougall and Biggs, 1952), in the presence of a base, usually calcium oxide or hydroxide. Mixing the oven-dried and ground plant tissue with a base prior to ashing avoids the possible loss of volatile boron esters through formation of nonvolatile borates at higher temperatures. Berger and Truog (1939) showed that even vegetative tissue higher in acidic constituents contains sufficient bases to control the loss of boron during ashing. Subsequently, McHargue and Hodgkiss (1941) substantiated this. Dible *et al.* (1954) did not add a base in the determination of boron in the vegetative tissue of alfalfa and timothy but advised addition of a base like calcium hydroxide prior to ashing, particularly in the case of oily seeds.

EXPERIMENTAL

The materials used were groundnut (peanut, *Arachis hypogaea* L.), TMV-2 (112 days old) and TMV-3 (142 days old) (Indian varieties). The plants were grown in pots, soil cultures from seeds supplied by the Regional Oilseeds Research Station, Kadiri (A.P.), India.

The plants were washed thoroughly with tap water and then with several fresh changes of boron-free deionized water. The pods were air-dried and the kernels collected. The plant samples and kernels were then dried in a steam oven. The vegetative parts of the plants were finely powdered in porcelain mortars. One-gram samples of large, medium, and small kernels were thoroughly crushed in porcelain crucibles. To one sample of each kernel size, 0.1 gram of boron-free calcium hydroxide was added and mixed well with a plastic spatula. The samples were ashed at 550° C. in an electric furnace and analyzed for boron by the simplified curcumin procedure (Dible *et al.*, 1954), using a Bausch and Lomb Spectronic 20 colorimeter. In another set of kernel samples, boron was estimated without the addition of $Ca(OH)_2$ before ashing. As before, boron was determined in the seeds of gingelly, castor, and Indian mustard (local). From the different vegetative parts of the groundnut plants, and in the mature middle leaves of some dicot and monocot crop plants (2 months old, raised in pots in soil), boron was estimated with and without the addition of calcium hydroxide [0.1 gram of $Ca(OH)_2$ per gram of oven-dried and finely powdered plant tissue] prior to ashing at 550° C. Similarly boron was determined in the mature middle leaves of six agricultural plants (collected from local cultivated fields).

RESULTS AND DISCUSSION

The results (Table I) of the determinations of boron in oilseeds clearly indicate that samples ashed without the addition of $Ca(OH)_2$ contained considerably less boron than the material treated with $Ca(OH)_2$ prior to ashing. Actually, the amount of boron in the samples with and without $Ca(OH)_2$ was the same but some was lost during ignition when $Ca(OH)_2$ was omitted—between 32.6 and 38.4%, calculated on the values of boron in samples ashed in the presence of a base.

In seeds, the possibility of the oil combining with boron to form a volatile borate ester accounts for the necessity of adding a base prior to ashing for boron determination. In the presence of $Ca(OH)_2$, a Ca borate forms, preventing boron loss on ignition.

Guevara R (1950) added 10% Na_2CO_3 to dried cereals, vegetables, and fruits before ashing, for boron estimation. As stated by Dible *et al.* (1954), and according to the present experimental results, addition of a base like $Ca(OH)_2$, prior to ashing, is apparently necessary if the boron content is to be accurately determined (without loss of boron during ignition at 550° C.). This is particularly true in seeds containing appreciable quantities of oil (groundnut, gingelly, castor, and Indian mustard) and may also be valid for other oily seeds.

The boron content of the different vegetative parts of groundnut plants and mature leaves of various plant species is given in Table II. It is clear that the addition of calcium hydroxide to the vegetative organs of the groundnut plants resulted in no significant difference in the amount of boron found. This was confirmed by the two treatments of the mature leaves of 11 other plant species.

Berger and Truog (1939) pointed out that vegetative tissues contain sufficient bases to prevent the loss of boron during ashing. Based on the results of recovery of boron added to vegetative tissue of different plant species, and the actual boron content obtained with or without the addition of CaO before ashing, Gupta (1967) concluded that the addition of CaO before ashing at 550° C. is not necessary. The determinations of boron in the present study also revealed that the addition of a base is not essential before ashing roots, stems, petioles, and leaves of plants at 550° C.

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Table I. Boron Content in Oilseeds of Four Plant Species[a]

(μg. per gram or p.p.m. on oven-dry basis)

Species	$Ca(OH)_2$ Added before Ashing	No $Ca(OH)_2$ Added before Ashing	% Loss of Boron in Material without $Ca(OH)_2$
Seed Material from Regional Oilseeds Research Station, Kadiri, (A.P.), India			
Groundnut (*Arachis hypogaea* L.)			
Variety TMV-2 (bunch type) kernels			
Large	8.4 ± 0.2	5.5 ± 0.3	34.5
Medium	7.9 ± 0.3	5.3 ± 0.1	32.9
Small	9.4 ± 0.1	6.1 ± 0.2	35.1
Variety TMV-3 (spreading type) kernels			
Large	10.4 ± 0.8	6.7 ± 0.4	35.6
Medium	12.3 ± 0.6	7.6 ± 0.5	38.2
Small	9.9 ± 0.3	6.3 ± 0.6	36.4
Seed Material from Pot Cultures			
Variety TMV-2 kernels			
Large	8.6 ± 0.1	5.7 ± 0.4	33.7
Medium	7.8 ± 0.2	5.1 ± 0.2	34.6
Small	9.2 ± 0.2	6.2 ± 0.3	32.6
Variety TMV-3 kernels			
Large	10.6 ± 0.4	6.8 ± 0.6	35.8
Medium	12.5 ± 0.7	7.7 ± 0.4	38.4
Small	9.8 ± 0.6	6.3 ± 0.5	35.7
Gingelly (*Sesamum indicum* L.) seeds	11.7 ± 1.1	7.8 ± 0.3	33.3
Castor (*Ricinus communis* L.) seeds	9.1 ± 0.7	6.1 ± 0.4	33.0
Indian mustard (*Brassica juncea* (L.) Czern. & Coss.) seeds	8.9 ± 0.3	5.7 ± 0.2	36.0

[a] Mean of 6 replications.

Table II. Boron Content of Different Agricultural Plants[a]

(μg. per gram or p.p.m. on oven-dry basis)

Plant	$Ca(OH)_2$ Added before Ashing	No $Ca(OH)_2$ Added before Ashing	% Difference of Boron in Material without $Ca(OH)_2$
Pot Cultures			
Groundnut (*Arachis hypogaea* L.) var. TMV-2			
Roots	18.9 ± 1.1	19.1 ± 1.3	+1.1
Stems	26.4 ± 2.3	26.2 ± 2.1	−0.8
Petioles	25.3 ± 2.4	25.6 ± 2.2	+1.2
Leaves	46.8 ± 3.6	47.0 ± 2.9	+0.4
Mature Leaves Collected from Middle Portion of Plants			
Groundnut var. TMV-3	41.5 ± 2.5	41.4 ± 3.1	−0.3
Tobacco (*Nicotiana rustica* L.)	36.6 ± 1.6	36.8 ± 2.2	+0.5
Lady's finger [*Abelmoschus esculentus* (L.) Moench]	43.2 ± 2.9	43.0 ± 3.2	−0.5
Rice (*Oryza sativa* L.)	4.9 ± 0.3	5.1 ± 0.2	+4.1
Jowar (*Sorghum vulgare* Pers.) var. G-2	5.3 ± 0.5	5.2 ± 0.3	−1.9
Onion (*Allium cepa* L.)	21.6 ± 1.1	21.8 ± 0.9	+0.9
Mature Leaves of Plants Cultivated in Local Fields			
Radish (*Raphanus sativus* L.)	86.3 ± 3.6	86.7 ± 3.3	+0.5
Tomato (*Lycopersicum esculentum* Mill.)	156.6 ± 5.8	156.9 ± 6.1	+0.2
Indian bean (*Dolichos lablab* L.)	48.2 ± 4.2	48.0 ± 2.3	−0.4
Maize (*Zea mays* L.)	5.2 ± 0.4	5.0 ± 0.3	−3.9
Sugarcane (*Saccaharum officinarum* L.)	10.5 ± 0.8	10.8 ± 0.6	+2.9
Plantain (*Musa paradisica* L.)	8.3 ± 0.6	8.2 ± 0.5	−1.2

[a] Mean of 6 replications.

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