Boron Isotope Ratios in Commercial Produce and Boron-10 Foliar and Hydroponic Enriched Plants

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Boron isotope ratios ($^{11}B/^{10}B$) for commercial produce ranged from a high of 4.162 ± 0.003 for cabbage to a low of 4.013 ± 0.008 for whole wheat flour. The observed isotope ratios for produce fall within the range reported for boron-containing minerals. Cucumbers and flour are ^{10}B enriched; bananas, cabbage, celery, grapes, green peppers, lettuce, oranges, potatoes, and tomatoes are ^{11}B enriched by at least 0.02; apples, broccoli, cantaloupe, and carrots are equal to NIST SRM-951 boric acid isotopic standard. Boron isotope ratios ($^{11}B/^{10}B$) were measured for broccoli and cabbage grown in a soilless medium, 4.018 ± 0.016 and 4.032 ± 0.003, in a soilless medium with foliar-applied $H_3{}^{10}BO_3$, 1.848 ± 0.009 and 1.746 ± 0.004, and in a hydroponic solution with $H_3{}^{10}BO_3$ as the only boron source, 0.126 ± 0.012 and 0.098 ± 0.005.

Variability found in an element's isotope ratio is defined as isotope fractionation. Isotope fractionation is known for elements with low atomic number and therefore large mass differences between isotopes. Fractionation occurs as a result of one of two processes: chemical reactions or physical processes (Faure, 1986). In covalent compounds, bond-breaking reaction rates are different for each isotope, resulting in the isotopic enrichment of reaction products. Examples of physical processes include evaporation, diffusion, and adsorption/desorption.

One example of fractionation is found in the fixation of CO_2 in plants. Carbon dioxide fractionation is the result of the different isotope reaction rates in different photosynthetic pathways (Kennedy and Krouse, 1991). Fractionation of CO_2 also occurs during diffusion into and out of plants. Calcium, an important biological element, fractionates in some industrial processes. Fractionation occurs during vacuum evaporation–distillation of calcium metal, CaF_2, or in ion-exchange columns with ionic calcium (Faure, 1986). Calcium has not been shown to fractionate in biological systems. This probably is the result of an ionic biochemistry that excludes fractionation through covalent bond-breaking reaction rates. Fractionation could possibly occur via an ion exchange or diffusion process, but this has not been reported.

Unlike the isotope ratios of many biologically important minerals, natural abundance boron isotope ratios are variable (Thode et al., 1948). Boron isotope ratios for geochemical systems (McMullen et al., 1961; Finley et al., 1962; Shima, 1963; Agyei and McMullen, 1968; Nomura et al., 1982; Swihart et al., 1986; Kakihana et al., 1987; Spivack and Edmond, 1987; Xiao et al., 1988; Musashi et al., 1990) have $^{11}B/^{10}B$ (‰ $\delta^{11}B$) [‰ $\delta^{11}B = ((R_{sample}/R_{standard}) - 1) \times 1000$, $R_{standard} = 4.04362 \pm 0.00137$, NIST SRM-951] ratios from 4.248 (+50.54) for borax to 3.815 (−50.5) for meteorites (Table I) (Shima, 1963). Analyses of boron-containing minerals have yielded $^{11}B/^{10}B$ (‰ $\delta^{11}B$) values ranging from 4.248 (+50.54) (Shima, 1963) to 3.922 (−30.08) (Finley et al., 1962).

Boron is known to fractionate under ion-exchange conditions (Kakihana et al., 1977). In nature, at low or intermediate temperatures, ocean bottom basalts bind boron. Since boron is presumed to be coordinated to basalts as $B(OH)_4^-$ (Spivack and Edmond, 1987) and the boron-isotope exchange reaction is not unity ($K = 1.0194$ at 298.1 K) (Kakihana et al., 1977)

$$^{10}B(OH)_3 + {}^{11}B(OH)_4^- = {}^{11}B(OH)_3 + {}^{10}B(OH_4)^-$$

the adsorbed $B(OH)_4^-$ is enriched with ^{10}B. This implies that boron may fractionate in biological systems given the proper conditions of concentration, temperature, pH, and reaction mechanism.

Preliminary data indicate that boron may be an essential trace element for mammals and suggest the importance of conducting human metabolism studies (Nielsen et al., 1987). Since boron lacks a suitable long-lived radioactive isotope, tracer studies require the use of stable boron isotopes. Boron isotope ratios have been performed for only a few biological samples, but no isotope absorption studies have been reported for animals, including humans. Boron isotopes have been used in isotope-dilution thermal ionization mass spectrometry (TIMS) measurements of boron in BCR-62 olive leaves, BCR-63 skimmed milk powder, NIST SRM-1571 orchard leaves (Duchateau et al., 1987), and BCR-281 ryegrass (Lamberty et al.,

Table I. Geochemical $^{11}B/^{10}B$ Isotope Ratios

year	author	material	$^{11}B/^{10}B$	‰ $\delta^{11}B$
1961	McMullen	borax and tincal	4.072–4.040	+7.02 to −0.89
1962	Finley	minerals	4.141–3.922	+2.41 to −30.08
1963	Shima	basalt	4.064–4.042	+5.04 to −0.40
		borax	4.248–4.240	+50.54 to +48.56
		gabbro	4.104–4.092	+14.93 to +11.96
		meteorites	3.960–3.815	−20.67 to −56.53
		seawater	4.071	+6.77
		slate	4.108–4.100	+15.92 to +13.94
		Tokyo Bay water	4.020	−5.84
1968	Agyei	gabbros	4.108–4.102	+15.92 to +14.44
		minerals	4.107–3.987	+15.67 to −14.0
		Tokyo Bay water	4.041	−0.62
1982	Nomura	fumarolic condensates	4.130–4.053	+21.36 to +2.32
		seawater	4.207–4.200	+40.40 to +38.67
1986	Swihart	evaporite borates	4.172–3.955	+31.75 to −21.92
1987	Kakihana	hot spring water	4.092–4.081	+11.96 to +9.24
1987	Spivack	seawater	4.2037	+39.5
		fresh basalts	4.04–4.03	−1.7 to −3.7
		altered basalts	4.08–4.04	+9.2 to −0.1
		hydrothermal	4.16–4.19	+36.8 to +30.0
		serpentinized peridotites	4.09–4.08	+12.6 to +8.3
1988	Xiao	borax	4.0305	−3.24
1990	Musaski	minerals	4.047–4.044	+0.84 to +0.09

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1988). Enriched boron labeling experiments have been done in a few plants; they include distribution of foliar-applied [^{10}B]boric acid in radishes (Chamel et al., 1981), white clover (Martini and Tellier, 1984), apples (Chamel and Andreani, 1985), *Lemna minor* (Thellier et al., 1979), and fruit trees (Hanson, 1991).

Since geochemical boron isotope ratios are variable, boron isotope ratios in both plants and animals should also vary to reflect their dietary boron sources (Kennedy and Krouse, 1990). This implies that human boron isotope absorption studies will require a basal boron isotope ratio measurement before the start of absorption studies and/or control of the boron isotope ratio through dietary manipulation.

To estimate the impact of diet on boron isotope ratios in human tissue, several variables are needed: daily boron intake, boron blood concentrations, and natural abundance ratios in humans and their diets. Boron isotope ratios in humans should be defined by diet, where the primary source of boron is fruits and vegetables (Nielsen, 1988). However, natural abundance boron isotope ratios have not been established for fruits or vegetables. Knowledge of boron isotope ratios in food will help us estimate the potential variation in human natural abundance boron isotope ratios. We have measured and are reporting boron isotope ratios in several commercially obtained food products.

Intrinsic vs extrinsic mineral utilization is one of the first questions addressed in biological isotope labeling studies. Isotope labeling studies for boron require both $H_3{}^{10}BO_3$ and plant material enriched with the boron-10 isotope. We have labeled plant materials by using both foliar and hydroponic techniques to compare boron-10 enrichments for use in future boron isotope studies.

MATERIALS AND METHODS

Reagents. Water was purified by a Super-Q system (distilled, deionized, 18 MΩ cm^{-1}; Millipore System, Super-Q, Millipore Corp., Bedford, MA) or a quartz subboiling (SB) still (Quartz Products Corp., Tuckerton, NJ) (Kuehner et al., 1972). Nitric (Baker, InstrAnalyzed) and hydrochloric (VWR, reagent grade) acids were purified by subboiling distillation. Six molar ammonia, from 58% ammonium hydroxide (VWR, reagent grade), was prepared by isothermal distillation (Veillon and Reamer, 1981). Cesium hydroxide (0.15 M) was prepared from CsCl (Alfa, 99.9999+%) by passing a cesium chloride solution through a 5-mL bed of IRA-743 (Amberlite IRA-743 boron-specific ion-exchange resin) and 15 g of AG 1-X8 (Bio-Rad, 20–50 mesh) ion-exchange resin in the hydroxide form (Spivack and Edmond, 1986). The IRA-743 resin was previously wet sieved to 60–100 mesh (Kiss, 1988). A 0.015 M $La(NO_3)_3$ solution (GFS, 99.999%) was prepared from $La(NO_3)_3{\cdot}6H_2O$, which was also passed through an IRA-743 column (Spivack and Edmond, 1986). A 0.3 M H_3BO_3 solution was prepared from NIST SRM-951 boric acid standard.

Equipment. All plasticware was initially soaked overnight in a Micro soap solution (International Products Corp., Trenton, NJ). Thereafter, plasticware was soaked 1–2 h in Micro, rinsed in demineralized water, and then soaked for 1–2 h in Radiac (Atomic Products Corp., Shirley, NY), rinsed in demineralized water, transferred to a 10% HNO_3 bath overnight, rinsed with SB distilled water, and dried in an oven at 50 °C.

Digestions were accomplished by use of a microwave digestion system (CEM Corp., Indian Trail, NC, MDS81D) (Schelkoph and Milne, 1988). A typical digestion used 0.4 g of sample, 5 mL of 16 M SB HNO_3, and 2 mL of 6 M HCl. After loading with sample and HNO_3, bombs were loosely capped and allowed to stand overnight. The next day SB HCl was added to each bomb, they were capped, and samples were digested for 12.5 min at 50% power, 5 min at 0% power, and 10 min at 75% power. Digests were analyzed by inductively coupled argon plasma emission spectroscopy (ICAP) on an ICP/6500 system (sequential analysis; Perkin-Elmer, Norwalk, CT) using operating conditions described previously (Hunt and Shuler, 1989).

Isolation. Boron was isolated from the digested matrix by Amberlite IRA-743 boron-specific ion-exchange resin (Aldrich) (Duchateau et al., 1987; Kiss, 1988). The only procedural changes from the literature (Kiss, 1988) included sample digestion with a microwave digestion system, overnight $(NH_4)_2C_2O_4$ precipitation of calcium (2 mL of 0.35 M), and passing of the digest four times through the IRA-743 columns. Boron was removed from the columns with 2 M HCl, and the solution volumes were reduced to ~5 μL in a dry bath at 60 °C. The resultant solutions were used for thermal ionization mass spectrometry. Boron recoveries were monitored by using NIST SRM-1572, citrus leaves (63.1 μg/g) (Iyengar et al., 1990), and typical recoveries were 60 μg/g.

Isotope ratio measurements were determined by using a Finnigan MAT 261 solid sample single-filament thermal ionization mass spectrometer. Isotope ratios were measured by using $Cs_2BO_2^+$ (Spivack and Edmond, 1986; Ramakumar et al., 1985) plus lanthanum nitrate added as an intensity enhancer (Zeininger and Heumann, 1983). A system standard was prepared by mixing 200 μL of 0.30 M H_3BO_3 (NIST SRM-951, boric acid), 200 μL of 0.15 M CsOH, 200 μL of 0.015 M $La(NO_3)_3$, and 400 μL of subboiling distilled water. This resulted in a solution with 0.06 M B, 0.03 M Cs, 0.003 M La, a B/Cs ratio of 2 (Spivack and Edmond, 1986), and a B/La ratio of 20 (Zeininger and Heumann, 1983). For the ~5-μL samples of produce digest, 5–10 μL of both 0.15 M CsOH and La^{3+} was required to generate satisfactory signal intensities.

Produce isotope ratios were converted from measured values (R) to true values (R') by $R = R'K$ (Duchateau et al., 1987). The bias factor, K, was calculated from the reported (4.04362) and observed (R_0) isotope ratio for the NIST SRM-951 system standard, $K = 4.04362/R_0$.

Commercial Produce. Produce in Table III was purchased (December 15, 1989) at a local market and cleaned as though it were to be eaten without cooking. The exception was Pillsbury whole wheat flour, which was used as purchased. All rinsing used subboiling water. Apples were rinsed, peeled, cored, and again rinsed. Bananas were peeled. Broccoli was rinsed, and the stems were trimmed, peeled, and rinsed again. Cabbage was rinsed, the stem was trimmed, and the top three layers of leaves were removed and again rinsed. Cantaloupe was rinsed and peeled, the seeds were removed, and the flesh was rinsed. Carrots were rinsed and peeled, both ends were trimmed, and the carrots were again rinsed. Celery was washed, both ends were trimmed, leaves were removed, and the stalks were again rinsed. Cucumbers were rinsed, peeled, and again rinsed. Grapes (green seedless) were rinsed and removed from the stem. Green peppers (sweet) were rinsed, the stem and seeds removed, and the peppers again rinsed. Lettuce (iceberg) was rinsed, trimmed, and cored. Oranges (navel) were rinsed and peeled. Tomatoes were rinsed and the stems removed.

Soilless Mix. Seeds were grown in 2-gal plastic pots containing Sunshine Mix 1, a soilless medium, with 48 g of Sierra control release fertilizer (13-12-11 and minor minerals, Sierra Chemical Co.). Plants were top dressed with ca. 20 g of Sierra fertilizer 2 months after planting.

Foliar. Isotopic enriched boron was applied to the plants grown in soilless mix at the beginning of the the third month after planting and then on a weekly basis for 1 month. Boron-10 boric acid ($H_3{}^{10}BO_3$) was foliar applied (Hanson et al., 1985) by spraying the top of the plant leaves until they dripped with a solution composed of 0.9152 g of $H_3{}^{10}BO_3$ (Eagle Picher, 95.91 at. %) and 0.529 g of Tween 80 (Sigma) dissolved in subboiling water and diluted to 500 mL. Solution pH was adjusted to 7.0 with 6 M KOH.

Hydroponics. Plants were grown in dark blue 3-gal square containers which were covered with black on white polyethylene, white side out for thermal control. After development of first leaves, plants were transferred from a germination pot of soilless mix to a nutrient solution composed of the primary solution listed in Table II (Chaney et al., 1989). All chemicals were Fisher Certified ACS grade except for $H_3{}^{10}BO_3$ (Eagle Picher) and $(NH_4)_6Mo_7)_{24}{\cdot}4H_2O$ (Aldrich). Mineral levels were maintained by making daily additions (Table II) using the following scheme: days 0–6, no addition; days 6–16, 5 mL of solution A and 0.1 mL of solution B; days 16–26, 10 mL of solution A and 0.2 mL of

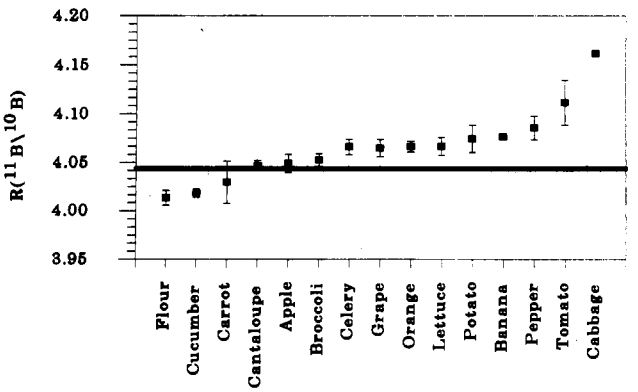

Figure 1. Boron isotope ratios found in commercially obtained produce. (■) Isotope ratio for corresponding produce; (—) 1σ limit; (horizontal bar) value in 1σ limit for NIST SRM-951, 4.04362 ● 0.00137.

Table II. Hydroponic Nutrient Solution

Primary Solution			
macro salts		micro salts	
KH_4PO_4,	0.01 mM	$H_3{}^{10}BO_3$,	10.0 μM
KNO_3,	2.44 mM	$MnCl_2{\cdot}4H_2O$,	1.0 μM
$Ca(NO_3)_2{\cdot}4H_2O$,	2.50 mM	$ZnSO_4{\cdot}7H_2O$,	5.0 μM
$MgSO_4{\cdot}7H_2O$,	1.00 mM	$CuSO_4{\cdot}5H_2O$,	1.0 μM
buffer		$(NH_4)_5Mo_7O_{24}{\cdot}$	
MES	2.0 mM	$4H_2O$,	0.2 μM
NaOH	1.0 mM	$CoCl_2{\cdot}6H_2O$,	0.2 μM
chelater		$NiCl_2{\cdot}6H_2O$,	0.5 μM
EDTA	127.7 μM	$Fe(NO_3)_3{\cdot}9H_2O$,	20.0 μM
KOH	0.319 mM	KCl,	46.6 μM
Daily Addition			
solution A		solution B	
KH_2PO_4,	5.0 μM	$H_3{}^{10}BO_3$,	89.67 nM
$(NH_4)_2SO_4$,	55.0 μM	$MnCl_2{\cdot}4H_2O$,	35.33 nM
$MgSO_4{\cdot}7H_2O$,	8.0 μM	$ZnSO_4{\cdot}7H_2O$,	29.67 nM
KCl,	14.83 μM		

solution B; day 26–end, 15 mL of solution A and 0.3 mL of solution B. Distilled water was added on a daily basis to maintain nutrient solution volume (boron concentration <15 ppb). Air, from an aquarium pump, was bubbled through each nutrient solution.

Plants used in the foliar and hydroponic study included broccoli (Premium Crop) and cabbage (Stonehead). Broccoli florets were harvested at the beginning of the fourth month after planting and cabbage half a month later. Material was harvested, processed, and frozen the same day. Broccoli florets were washed in Radiac and then rinsed in distilled water (Hanson et al., 1985). The outer three leaf layers were removed from the harvested cabbage heads, and the heads were then rinsed in distilled water. All material was placed into ziplock bags, frozen, and freeze-dried for analysis.

RESULTS AND DISCUSSION

Boron isotope ratios ($^{11}B/^{10}B$ (‰ $\delta^{11}B$)) for commercial produce (Table II) ranged from a high of 4.162 (+29.27) for cabbage to a low of 4.013 (−7.50) for whole wheat flour (Figure 1). The average isotope ratio for produce reported in Table II is 4.066 (+5.53). The observed boron isotope ratios for produce fall within the range reported (see introduction; Table I) for geochemical isotope ratios in general (4.248–3.815) and specifically for boron-containing minerals (4.248–3.922). Cucumber and flour (2 of 15 samples) are enriched in ^{10}B compared to SRM-951 (Figure 1), while apples, broccoli, cantaloupe, and carrots (4 of 15 samples) nearly equal the reference value. Compared to SRM-951, other produce ratios (9 of 15 samples) are enriched in ^{11}B by at least 0.02 (−4.95). The variability in 1σ for the isotope ratios in Table II reflects differences in sample preparation, analytical variability, and sample inhomogeneity.

We have not attempted to correlate produce boron isotope ratios to geological or soil boron isotope ratios. This would have required obtaining soil samples from the produce production location for each of the foods analyzed. While soil boron must contribute to ratios found in commercial produce, other boron sources may also contribute, or even define, boron isotope ratios in produce. Major sources of boron could include the water supply and commercial fertilizers.

Almost all dietary boron is excreted in urine (98%, 550 μg/day), while only a trace appears in fecal material (2%, 14 μg/day) (Tipton et al., 1966). Therefore, as a minimum requirement, boron must be transferred to blood plasma before urinary excretion. Total human blood volume is estimated to be 3–6 L (Altman and Dittmer, 1974) (mean of 4.5 L) with the low volume for small women and the high volume for large men. Daily boron intakes have been estimated to range from 0.5 to 3.1 mg (Nielsen, 1990) (mean of 1.8 mg/day) and total blood boron concentrations range from 15.3 to 79.5 ng/g of wet weight (Clarke et al., 1987) (mean of 47.4 ng/g).

Using average values for blood volume and blood boron concentration, total blood boron is roughly 213 μg. Therefore, expressing average total daily boron intake (1800 μg/day) relative to total blood boron gives a "daily intake/total blood boron" ratio of 8.5. This estimate would indicate that the boron isotope ratio for blood, or at least plasma, could be defined by the daily boron intake. Blood boron levels might be transiently defined by a single meal or highly influenced by a snack. For example, one medium banana (119 g) (Guthrie, 1983) with 5.8 μg of B/g of wet weight (Table II) could contribute 690 μg of boron. Using average values, this potentially represents a 3:1 ratio of intake to total blood boron.

If we assume a human natural abundance boron isotope ratio of 4.04362 and an average total blood boron value of 213 μg, a banana with an isotope ratio of 4.0764 could change the human boron isotope ratio in blood to 4.0686 (‰ $\delta^{11}B = +6.18$), a change of 0.025 (0.62%). Consumption of a single tomato could produce even larger changes in blood boron isotope ratios (Table III). In response to the total daily boron intake and the isotope ratio associated with each food, human isotope ratios could change throughout the day. Isotope ratios in human tissues could also fluctuate seasonally with changes in intake of fresh fruits and vegetables or changes in the proportion of locally produced vs imported products consumed. However, the above estimates could be moderated by a rapid exchange between blood boron and other body boron pools.

Variable boron isotope ratios and their potential effect on blood isotope ratios impose some severe restrictions on isotope tracer studies in humans. These are most apparent as an intrinsic/extrinsic study. The most obvious solution is to grow food products highly or 100% enriched with ^{10}B by using foliar or hydroponic techniques.

Broccoli and cabbage plants were raised to maturity in a greenhouse utilizing (1) a soilless medium with a commercial fertilizer (labeled soilless mix in Table IV), (2) the same as (1), plus a foliar $H_3{}^{10}BO_3$ leaf spray (labeled foliar in Table IV), and (3) a hydroponic nutrient solution with $H_3{}^{10}BO_3$ as the only major boron source.

Broccoli and cabbage grown in soilless mix with commercial fertilizer had boron isotope ratios of 4.018 ± 16 and 4.032 ± 3, respectively (Table IV). Identical plantings of broccoli and cabbage, with foliar-applied boron-10 during broccoli floret and cabbage head formation, resulted in respective boron isotope ratios of 1.848 ± 9 and 1.746 ± 4. The high broccoli wet weight boron concentration may reflect inadequate removal of foliar-applied boron, so intrinsic boron isotope ratios may not be accurately

Table III. Boron Levels and $^{11}B/^{10}B$ Isotope Ratios in Commercial Produce

produce	growth source	μg of B/g ±1σ dry wt	μg of B/g ±1σ wet wt	n	$^{11}B/^{10}B$, ±1σ	‰ $\delta^{11}B$	n
apples	Washington	42.5 ± 3	6.44 ± 4	2	4.049 ± 9	+1.33	7
bananas	Guatemala	20.6 ± 6	5.8 ± 2	2	4.0764 ± 2	+8.11	3
broccoli	California	21.9 ± 7	2.37 ± 8	3	4.053 ± 6	+2.32	3
cabbage	Texas	18.3 ± 3	1.23 ± 3	3	4.162 ± 3	+29.27	5
cantaloupe	California	16.0 ± 7	1.61 ± 7	3	4.047 ± 5	+0.84	9
carrots	Minnesota	13.9 ± 8	1.56 ± 9	3	4.030 ± 22	−3.61	8
celery	California	24.7 ± 4	1.36 ± 2	2	4.066 ± 8	+5.53	3
cucumber	California	20 ± 2	0.65 ± 6	2	4.018 ± 4	−6.34	4
flour, whole wheat	Pillsbury	7.3 ± 2		2	4.013 ± 8	−7.50	1
grapes	Chile	27.2 ± 6	5.0 ± 1	2	4.065 ± 9	+5.24	3
green peppers	California	8.6 ± 2	0.55 ± 1	2	4.086 ± 12	+8.75	5
lettuce	California	19.8 ± 9	0.76 ± 3	3	4.066 ± 9	+5.51	3
oranges	California	13.2 ± 4	1.98 ± 6	3	4.066 ± 6	+5.61	3
potatoes	North Dakota	1.5 ± 1	0.28 ± 2	3	4.074 ± 14	+7.51	2
tomato	Mexico	27.1 ± 7	1.54 ± 4	2	4.112 ± 23	+16.91	9

Table IV. Boron Concentrations and $^{11}B/^{10}B$ Isotope Ratios in Broccoli and Cabbage

produce	location	μg of B/g, ±1σ dry wt	μg of B/g, ±1σ wet wt	n	$^{11}B/^{10}B$, ±1σ	‰ $\delta^{11}B$	n
broccoli	commercial	21.9 ± 7	2.37 ± 8	3	4.053 ± 6	+2.32	3
	soilless mix	31.4 ± 7	2.17 ± 5	3	4.018 ± 16	−6.34	5
	foliar	31.5 ± 5	4.41 ± 6[a]	2	1.848 ± 9	−543	5
	hydroponic	24.3 ± 3	2.67 ± 4	2	0.126 ± 12	−969	4
cabbage	commercial	18.3 ± 5	1.23 ± 3	3	4.162 ± 3	+29.3	5
	soilless mix	15.5 ± 8	1.57 ± 9	3	4.032 ± 3	−2.87	3
	foliar	19.5 ± 4	2.05 ± 4	3	1.746 ± 4	−568	4
	hydroponic	13 ± 2	1.3 ± 2	3	0.098 ± 5	−976	6

[a] High broccoli wet weight boron concentrations reflect inadequate removal of foliar-applied boron.

determined (Table IV). A similar argument could be made for cabbage. However, removal of the top three leaf layers makes contamination unlikely. Since care was taken in the foliar spray application, the higher wet weight boron levels may be intrinsic boron levels.

While broccoli and cabbage were grown under nearly identical hydroponic conditions, the broccoli boron concentration is nearly twice that of cabbage (2.67 vs 1.3 μg of B/g of wet weight, Table IV). However, for all measured sources (Table IV), broccoli boron concentrations are about twice for those determined for cabbage. Low boron concentrations in cabbage probably result from measurement of the inner storage leaves and not the discarded outer leaves.

On the basis of the 95.91 at. % enriched boron-10 boric acid, hydroponic grown material should give a theoretical $^{11}B/^{10}B$ ratio of 0.0426. Boron isotope ratios ($^{11}B/^{10}B$) measured in hydroponic grown broccoli florets and cabbage head were 0.1281 ± 12 and 0.098 ± 5, respectively. There are two possible explanations for the divergence from theoretical values. First, boron contamination in the distilled water used to prepare the hydroponic solutions would modify the expected isotope ratio in both the nutrient solution and the growing plant. Second, the plants were grown during the summer next to drip walls for cooling. These walls allowed dust and dirt into the greenhouse and into the hydroponic solutions, which could also raise the expected isotope ratio. Since the boron concentrations in the hydroponic solution are low, contamination could make a significant contribution to the solution's boron content and isotope ratio.

An isotope ratio change of 0.5 is easily measured in biological samples through use of either thermal ionization or inductively coupled mass spectrometry. On the basis of previously discussed values for humans (213 μg of TBB, R = 4.04362) and data in Table IV for hydroponically grown broccoli (24.3 μg of B/g, 9.10 g wet/g dry, R = 0.126), an estimate of human plasma enrichment for a given broccoli meal may be calculated. To achieve a hypothetical plasma isotope ratio change of ΔR = 0.5, 2.366 g of wet broccoli may be required. For a typical serving of broccoli, 1 cup or 150 g (Guthrie, 1983), the plasma isotope ratio could change as much as ΔR = 3.525 to R = 0.518. Obviously, actual changes in blood plasma isotope ratios could be modified by factors such as time of last meal, boron concentration in last meal, rate of absorption of the ^{10}B spike, rate of natural abundance blood boron excretion, and rate of ^{10}B transport to other tissues.

A large boron enrichment was observed in both the foliar and hydroponic grown plants. The enrichment is certainly larger than would be obtained through soil supplementation or stem injection of $H_3{}^{10}BO_3$. Either enrichment product would be satisfactory for an intrinsic/extrinsic utilization study as they were both very high. However, because of the variability in natural abundance boron isotope ratios and potential low retention (vs absorption) in humans, hydroponic enrichment plant material may be required for intrinsic/extrinsic experiments in humans.

Summary. Boron isotope ratios in commercial produce are variable. Based on typical daily boron intake, and as a lower limit, a single serving of fruits or vegetables may contribute 50% or more to an individual's total blood boron. At higher intakes, a serving may define the blood (plasma) isotope ratio. In both cases the influence on human boron isotope ratios should be measurable. Human nutrition research and boron isotope studies will, therefore, require strict accounting of boron isotope ratios in the diet or very high isotope incorporation in an isotope labeling study. Isotope tracer studies in laboratory animals should be somewhat easier because animals can be fed a semisynthetic diet of constant composition for long periods of time. Boron-10 enriched broccoli and cabbage have been raised for potential intrinsic/extrinsic studies. Boron-10 enrichments are possible with foliar sprays, even with plants adequately supplied with boron, but hydroponic grown plants yield the highest boron-10 levels. However, for plant material to be considered intrinsically labeled,

the external foliar spray has to be completely removed from the surface of the plant. This was successful in the case of cabbage and potentially unsuccessful for broccoli, most likely due to the nature of the floret's surface.

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