# Vitamin B6 and Pyridoxine Glucoside Content of Wheat and Wheat Flours

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## ABSTRACT

Analysis of different wheats and flours for vitamin B6 using water extracts and reverse-phase high-performance liquid chromatography revealed significant variation in content of pyridoxine (PN) and pyridoxine glucoside (PNG), a glycosidic adduct of B6 with partial human bioavailability found in many plant foods. Other vitameric forms of vitamin B6 were not detected. In 22 American and Canadian wheats, total vitamin B6 (the sum of PN plus PNG observed in samples) content varied over a threefold range of 3.4 to 11.1 nmol/g. PNG accounted for a significant portion of the variation (1.9–8.8 nmol/g), with less variation in PN (0.9–2.7 nmol/g). Mean values (± standard deviations) were 5.0 ± 1.8 nmol/g for total vitamin B6; 1.7 ± 0.5 nmol/g for PN; and 3.4 ± 1.8 nmol/g for PNG. American hard wheats contained significantly more vitamin B6, mostly as PNG, compared to Canadian hard wheats. The same pattern was seen for Colorado hard wheats compared to others. Hard wheats contained more vitamin B6 than did soft wheats. Flours milled from 16 of the cultivars contained ≈75% less B6 than did the corresponding wheats.


The U.S. Food and Nutrition Board suggested in the 1989 Recommended Daily Allowances (RDA) (National Research Council 1989) that whole-wheat foods are a good source of vitamin B6 in the American diet. However, a significant and variable fraction of vitamin B6 in plant foods, including wheat, is present as pyridoxine glucoside (5′-O-(B-D-glucopyranosyl)pyridoxine) (PNG) (Gregory and Ink 1987), which has partial bioavailability for humans (Gregory et al 1991). Many Americans consume vitamin B6 at levels significantly less than the current RDA (Kant and Block 1990).

Vitamin B6 includes a group of 3-hydroxy-2-methyl-pyridine derivatives that can exist in the C-4′ position as an alcohol (pyridoxine) (PN), an aldehyde (pyridoxal) (PL) and an amine (pyridoxamine) (PM). These vitameric forms can be phosphorylated or nonphosphorylated. The available data suggest that PN predominates in most plant foods (Van Schoonhoven et al 1994). However, most of the reports in the literature have used a microbiological procedure involving an acid hydrolysis step during sample extraction (Toepfer and Lehmann 1961). This procedure converts any PNG in the sample into PN (Gregory 1988). Therefore, in much of the literature about vitamin B6 in plant foods, PNG present in the food may be reported as PN.

We recently reported an high-performance liquid chromatography (HPLC) method capable of measuring vitamin B6 and PNG in wheat (Sampson et al 1995). Although the method does not detect quantitatively minor forms of vitamin B6 such as polyglycosylated adducts (Gregory and Sartain 1991), this method is simple, quick and reliable, and does detect the major forms of vitamin B6 in wheat, including PN and PNG. Our initial analyses of vitamin B6 in several wheat cultivars obtained with this method showed that 36–81% of vitamin B6 in several wheat cultivars was present as PNG (Sampson et al 1995). We have now used this method to investigate the vitamin B6 content of American and Canadian wheats and wheat flours.

Our objectives in this study were: 1) to evaluate the vitamin B6 content, including PNG, of various cultivars of American and Canadian wheat; 2) to determine whether differences in these vitamers occurred between American and Canadian strains and between hard vs. soft wheats; 3) to compare wheats grown in Colorado to those from other American and Canadian locations; and 4) to determine the extent of loss of these compounds associated with commercial milling. Our interest in American vs. Canadian and Colorado vs. other wheats stemmed from the importance of wheat to Colorado agriculture.

## MATERIALS AND METHODS

### Reagents

Vitamin B6 standards, deoxypyridoxine, and HPLC reagents were obtained from vendors described earlier (Sampson et al 1995).

### Sample Preparation

Seventeen hard wheats and 5 soft wheats, as well as flours milled from 16 of these wheats, were obtained from commercial mills in the United States and Canada. Information about milling, patent percentage, and origin of cultivars was obtained from the mills. Wheats were stored at –20°C in 100-ml polycarbonate bottles before analysis. Sample preparation was done under yellow fluorescent lighting. Samples (10 g) were prepared in triplicate as described previously (Sampson et al 1995) using a micro-mill (Lab Apparatus, Cleveland, OH) and homogenizer (Hi-Speed, model 45, Virtis Co., Gardiner, NY). Moisture, protein, and ash contents of the wheats and flours were determined using approved methods (AACC 1995). Protein was expressed as N × 6.25 for grain and as N × 5.7 for flours.

### HPLC Equipment and Method

Wheats were analyzed by reverse-phase HPLC using gradient elution and fluorescence detection, as described elsewhere (Sampson et al 1995). Mobile phase A of the binary gradient contained 0.033*M* phosphoric acid and 0.008*M* 1-octanesulfonic acid at pH 2.2. Mobile phase B contained 0.033*M* phosphoric acid and 10% (v/v) acetonitrile at pH 2.2. Fluorescence was enhanced by postcolumn derivitization with sodium bisulfite. The binary gradient involved a linear ramp from 100% A to 100% B in 10 min, 15 min in 100% B, ramp back to 100% A in 4.5 min, and 5.5 min in 100% A. PN and PNG were quantified relative to fluorescence of the internal standard, deoxypyridoxine. Molar fluorescence of PNG was assumed to be equivalent to PN, as shown by Gregory and Ink (1987).

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**TABLE I**
**Identification and Proximate Composition of U.S. and Canadian Grains and Flours**

| Sample No. or Variety[a] | Wheat Type[b] | Origin[c] | Grain Protein[d] (%) | Flour Extraction (%) | Flour Patent (%) | Flour Type[e] | Flour Protein[d] (%) | Flour Ash[d] (%) |
|---|---|---|---|---|---|---|---|---|
| 86 | WRS | Unknown | 13.1 | 72.0 | 59.0 | B | 12.9 | 0.47 |
| 81 | WRS | AB | 12.5 | 73.0 | 80.0 | F | 12.2 | 0.47 |
| 73 | WRS | AB | 15.1 | 76.0 | ... | S | 14.4 | 0.48 |
| 34 | HW | ID | 11.7 | 70.0 | 100.0 | F | 10.4 | 0.43 |
| 70 | ARS | AB | 13.1 | 75.5 | ... | B | 12.8 | 0.48 |
| 87 | WRS | MB | 12.8 | 75.5 | 71.5 | B | 12.5 | 0.50 |
| 39 | HS | ID | 12.8 | ... | 71.6 | M | 11.6 | 0.42 |
| 83 | WRS | Unknown | 13.1 | 73.0 | 52.0 | F | 11.7 | 0.38 |
| 40 | HS | ND | 13.6 | 73.0 | 98.0 | B | 12.6 | 0.51 |
| 11 | HW | MN | 10.9 | 74.1 | 96.8 | F | 9.9 | 0.41 |
| Colano | HS | CO | 15.8 | ... | ... | ... | ... | ... |
| 42 | HS | ND | 14.6 | 75.0 | 100.0 | H | 13.8 | 0.54 |
| Waldron | HW | CO | 12.8 | ... | ... | ... | ... | ... |
| 46 | HS | MN | 15.0 | 74.5 | 100.0 | H | 13.9 | 0.58 |
| Vona | HW | CO | 10.6 | ... | ... | ... | ... | ... |
| Scout | HW | CO | 12.0 | ... | ... | ... | ... | ... |
| TAM 107 | HW | CO | 10.7 | ... | ... | ... | ... | ... |
| 79 | OSW | ON | 10.1 | 70.0 | 100.0 | S | 9.1 | 0.50 |
| 76 | OSW | ON | 10.4 | 75.0 | 100.0 | C | 8.1 | 0.36 |
| 33 | SW | ID | 10.2 | 75.7 | 50.3 | C | 7.9 | 0.35 |
| 56 | S | WA | 9.6 | 70.5 | 58.5 | C | 7.7 | 0.38 |
| 31 | SRW | TN | 9.2 | 75.0 | 82.0 | F | nd | nd |

[a] Data in Tables I, II, and III are presented in the same order to facilitate comparison.
[b] HW = hard red winter; SW = soft white; SRW = soft red winter; HS = hard red spring; ARS = Alberta red spring; OSW = Ontario soft winter; WRS = western red spring.
[c] MN = Minnesota; ID = Idaho; ND = North Dakota; WA = Washington; TN = Tennessee; AB = Alberta; ON = Ontario; MB = Manitoba; CO = Colorado.
[d] 14% moisture basis; nd = not determined.
[e] F = family; C = cake; M = macaroni; H = hearth bread; B = baker's bread flour; S = cookie, crackers

**TABLE II**
**Vitamin B6 Concentrations (nmol/g) in Whole Wheats[a,b]**

| Type[c] | Sample Country[d] | Sample Locale[e] | PN | PNG | Total B6 |
|---|---|---|---|---|---|
| Hard wheats | | | | | |
| WRS | CAN | Unknown | 0.87 ± 0.04 | 2.52 ± 0.08 | 3.39 ± 0.07 |
| WRS | CAN | AB | 0.99 ± 0.07 | 2.63 ± 0.28 | 3.62 ± 0.21 |
| WRS[f] | CAN | AB | 1.36 ± 0.12 | 2.58 ± 0.07 | 3.94 ± 0.19 |
| HW | USA | ID | 1.31 ± 0.15 | 2.68 ± 0.10 | 3.99 ± 0.19 |
| ARS | CAN | AB | 1.35 ± 0.12 | 2.74 ± 0.34 | 4.09 ± 0.39 |
| WRS | CAN | MB | 1.51 ± 0.22 | 2.63 ± 0.20 | 4.14 ± 0.40 |
| HS[f] | USA | ID | 1.88 ± 0.06 | 2.34 ± 0.14 | 4.22 ± 0.12 |
| WRS | CAN | Unknown | 2.66 ± 0.15 | 1.80 ± 0.08 | 4.46 ± 0.22 |
| HS | USA | ND | 2.70 ± 0.01 | 1.88 ± 0.23 | 4.58 ± 0.23 |
| HW[f] | USA | MN | 1.23 ± 0.09 | 4.10 ± 0.20 | 5.33 ± 0.29 |
| HS | USA | CO | 1.68 ± 0.05 | 3.72 ± 0.19 | 5.40 ± 0.18 |
| HS | USA | ND | 1.92 ± 0.23 | 3.89 ± 0.52 | 5.81 ± 0.75 |
| HW | USA | CO | 1.86 ± 0.02 | 4.37 ± 0.26 | 6.23 ± 0.27 |
| HS | USA | MN | 2.38 ± 0.26 | 4.06 ± 0.13 | 6.44 ± 0.39 |
| HW | USA | CO | 2.10 ± 0.27 | 4.88 ± 0.40 | 6.98 ± 0.55 |
| HW | USA | CO | 1.69 ± 0.02 | 5.43 ± 0.30 | 7.12 ± 0.29 |
| HW | USA | CO | 2.34 ± 0.22 | 8.76 ± 0.34 | 11.10 ± 0.56 |
| Soft wheats | | | | | |
| OSW | CAN | ON | 1.10 ± 0.04 | 1.97 ± 0.19 | 3.07 ± 0.17 |
| OSW | CAN | ON | 1.36 ± 0.12 | 1.88 ± 0.02 | 3.24 ± 0.11 |
| SW | USA | ID | 1.39 ± 0.25 | 3.09 ± 0.10 | 4.48 ± 0.35 |
| S | USA | WA | 1.02 ± 0.14 | 3.56 ± 0.04 | 4.58 ± 0.18 |
| SRW | USA | TN | 2.25 ± 0.06 | 2.34 ± 0.03 | 4.59 ± 0.09 |

[a] Values are mean ± standard deviations for $n = 3$ replicates, except as indicated. Data are presented in order of increasing total vitamin B6. Data in Tables I, II, and III are presented in the same order to facilitate comparison.
[b] PN = pyridoxine; PNG = pyridoxine glucoside; Total B6 = sum of PN + PNG (other vitamins not detected).
[c] WRS = western red spring; HW = hard red winter; ARS = Alberta red spring; HS = hard red spring; OSW = Ontario soft winter; SW = soft white; S = soft; SRW = soft red winter.
[d] CAN = Canada; USA =United States.
[e] AB = Alberta; ID = Idaho; MB = Manitoba; ND = North Dakota; MN = Minnesota; CO = Colorado; ON = Ontario; WA = Washington; TN = Tennessee.
[f] $n = 2$ replicates.

## RESULTS

Sample identification and proximate composition of whole-grains and flours is given in Table I. Percent protein ranged from 9.2 to 15.1% and did not correlate with content of vitamin B6 in the wheats analyzed.

All of the wheats analyzed contained vitamin B6 as PN and PNG, with none of the other B6 vitamers (PL, PLP, PM, PMP) detected by this method. Limits of detection, expressed as signal-to-noise ratio = 3, were ≈0.07–0.29 pmol per 20 µl injection for the different B6 vitamers, corresponding to 0.02–0.07 nmol/g sample. Therefore PL, PLP, PM, and PMP in these samples were present at levels <0.07 nmol/g. Total vitamin B6 will refer to the sum of PN plus PNG observed in samples.

There was significant variation in content of PN and PNG (Table II). Observed total vitamin B6 content varied over a three-fold range (3.4–11.1 nmol/g) associated with a smaller variation in PN (0.9–2.7 nmol/g) and a larger variation in PNG (1.9–8.8 nmol/g). The means ± standard deviations for these ranges were: 5.0±1.8 nmol/g, total vitamin B6; 1.7 ± 0.5 nmol/g, PN; 3.4 ± 1.8 nmol/g, PNG. Most cultivars contained vitamin B6 in a range of 3.4–7.1 nmol/g for total B6 and 1.9–5.4 nmol/g for PNG. One American cultivar (HW, USA, CO) contained significantly more vitamin B6 than other cultivars, with 11.1 nmol/g for total B6 and 8.8 nmol/g for PNG. The contributions of PN and PNG to total vitamin B6 in these wheats varied in lower vs. higher B6-containing cultivars. In cultivars containing total vitamin B6 in the range of 3–5 nmol/g, samples with higher totals had more PN. In contrast, in cultivars containing vitamin B6 in the range of 5–11 nmol/g, samples with higher totals had more PNG.

American wheats (11 cultivars) showed a wide range of total vitamin B6 content (4.0–11.1 nmol/g for total vitamin B6) in contrast to Canadian wheats (six cultivars), which covered a narrower range of 3.4–4.5 nmol/g for total vitamin B6 (Table II). On average, the American cultivars contained 56% more total vitamin B6 than did those from Canada (6.1 ± 2.0 vs. 3.9 ± 0.4 nmol/g, respectively). Most of this difference was due to higher PNG content, which averaged 68% higher in the American wheats when compared to Canadian wheats (4.2 ± 1.9 vs. 2.5 ± 0.3 nmol/g, respectively). This differences between American and Canadian wheats remained even when the American cultivar with particularly high total vitamin B6 (HW, USA, CO) was removed from analysis, although the average percent differences for total vitamin B6 and PNG decreased to +44% and +48%, respectively.

The total vitamin B6 and PNG content tended to be higher in hard wheats than in soft wheats (Table II). For hard wheats, the range (and mean ± standard deviation) were: 3.4–11.1 nmol/g (5.3 ± 1.9 nmol/g) for total vitamin B6; 0.9–2.7 nmol/g (1.8 ± 0.6 nmol/g) for PN; and 1.8–8.8 nmol/g (4.0 ± 1.7 nmol/g) for PNG. The corresponding values for soft wheats were: 3.1–4.6 nmol/g (4.0 ± 0.8 nmol/g) for total vitamin B6; 1.0–2.2 nmol/g (1.4 ± 0.5 nmol/g) for PN; and 1.9–3.6 nmol/g (2.6 ± 0.7 nmol/g) for PNG. Thus, on average, hard wheats contained 32% more total vitamin B6 than did soft wheats, accounted for primarily by 54% more PNG.

Compared to other hard wheats, those from Colorado (five cultivars) contained 61% more total vitamin B6 (7.4 ± 2.2 vs. 4.6 ± 0.9 nmol/g) comprised primarily of 80% more PNG (5.4 ± 2.0 vs. 3.0 ± 0.8 nmol/g), with little difference between PN content (1.9 ± 0.3 vs. 1.7 ± 0.6 nmol/g). Four out of five cultivars with highest total B6 and PNG content were from Colorado. The wheat cultivar containing the highest content of total B6 as well as PNG is one of two most widely grown wheats in Colorado (TAM-107). The other widely grown Colorado wheat (Vona) had similarly high total B6 and PNG (7.0 ± 0.6 nmol/g for total B6).

Milling whole wheats into flours removed significant amounts of vitamin B6 (Tables III vs. II). For 12 flours prepared from hard wheats and four from soft wheats, the average loss of total vitamin B6 from milling was -74% (4.3±0.9 vs. 1.1±0.4 nmol/g). Percent losses were somewhat higher for PNG (–79%) (2.8 ± 0.8 vs. 0.6 ± 0.2 nmol/g) than for PN (–69%) 1.6 ± 0.6 vs. 0.5 ± 0.2 nmol/g). For two cultivars with low patent percent (50–52%), the loss in PN for flour vs. whole wheat (–81 ± 1%) was significantly higher than for 12 cultivars with higher patent percent (58–100%) (–63 ± 6%).

## DISCUSSION

The main conclusion from our data is that wheats from the United States and Canada contain easily extractable vitamin B6 in a range from 3 to 11 nmol/g, and that flours from those wheats contain ≈1 nmol of total vitamin B6/g. Most reports in the litera-

**TABLE III**
**Vitamin B6 Concentrations (nmol/g) in Wheat Flours[a,b]**

| | Sample | | | | |
|---|---|---|---|---|---|
| Type[c] | Country[d] | Locale[e] | PN | PNG | Total B6 |
| Hard wheats | | | | | |
| WRS | CAN | Unknown | 0.34 ± 0.01 | 0.52 ± 0.05 | 0.85 ± 0.06 |
| WRS | CAN | AB | 0.33 ± 0.02 | 0.51 ± 0.07 | 0.84 ± 0.09 |
| WRS[f] | CAN | AB | 0.49 ± 0.04 | 0.42 ± 0.06 | 0.91 ± 0.08 |
| HW | USA | ID | 0.43 ± 0.03 | 0.71 ± 0.04 | 1.14 ± 0.06 |
| ARS | CAN | AB | 0.49 ± 0.04 | 0.67 ± 0.01 | 1.16 ± 0.04 |
| WRS | CAN | MB | 0.36 ± 0.01 | 0.90 ± 0.05 | 1.26 ± 0.06 |
| HS[f] | USA | ID | 0.82 ± 0.03 | 0.28 ± 0.04 | 1.10 ± 0.06 |
| WRS | CAN | Unknown | 0.47 ± 0.04 | 0.53 ± 0.03 | 1.00 ± 0.07 |
| HS | USA | ND | 1.04 ± 0.24 | 0.77 ± 0.12 | 1.81 ± 0.29 |
| HW[f] | USA | MN | 0.51 ± 0.01 | 0.38 ± 0.04 | 0.89 ± 0.04 |
| HS | USA | ND | 0.64 ± 0.02 | 0.96 ± 0.02 | 1.61 ± 0.04 |
| HS | USA | MN | 0.88 ± 0.07 | 0.70 ± 0.06 | 1.57 ± 0.02 |
| Soft wheats | | | | | |
| OSW | CAN | ON | 0.47 ± 0.05 | 0.71 ± 0.05 | 1.18 ± 0.04 |
| OSW | CAN | ON | 0.47 ± 0.01 | 0.33 ± 0.05 | 0.80 ± 0.05 |
| SW | USA | ID | 0.27 ± 0.03 | 0.12 ± 0.03 | 0.39 ± 0.06 |
| S | USA | WA | 0.43 ± 0.04 | 0.35 ± 0.03 | 0.79 ± 0.07 |

[a] Values are mean ± standard deviations for $n = 3$ replicates, except as indicated. Data in Tables I, II, and III are presented in the same order to facilitate comparison.
[b] PN = pyridoxine; PNG = pyridoxine glucoside; Total B6 = sum of PN + PNG (other vitamins not detected).
[c] WRS = western red spring; HW = hard red winter; ARS = Alberta red spring; HS = hard red spring; OSW = Ontario soft winter; SW = soft white; S = soft.
[d] CAN = Canada; USA =United States.
[e] AB = Alberta; ID = Idaho; MB = Manitoba; ND = North Dakota; MN = Minnesota; CO = Colorado; ON = Ontario; WA = Washington.
[f] $n = 2$ replicates.

ture suggest that whole wheat contains ≈23 nmol/g for vitamin B6, mostly present as PN (≈16 nmol/g), with lesser amounts of PL and PM (2–4 nmol/g, each) (Toepfer and Lehmann 1961, Polansky et al 1964, Toepfer and Polansky 1970, Toepfer et al 1972, Michaela and Lorenz 1976, Keagy et al 1980, Vanderslice et al 1984). Whole wheat flour has been reported to contain ≈19 nmol/g for vitamin B6, present primarily as PN (15 nmol/g) (Toepfer and Polansky 1964, 1970; Michaela and Lorenz 1976; Kabir et al 1983). Two reports show that white flour contains much lower amounts of vitamin B6 (2–4 nmol/g) (Michaela and Lorenz 1976, Keagy et al 1980).

That our vitamin B6 values for wheat are lower than in earlier reports raises the possibility of incomplete extraction using our method. However, we reported previously that our method gives complete extraction of PN, measured using exogenous PN spikes. We have assumed that PNG recovery is similarly high. Two other laboratories have reported that PNG extraction from plant foods is as high as that for PN (Gregory and Feldstein 1985, Tadera et al 1986, Gregory and Ink 1987, Tadera and Naka 1991). Gregory (Gregory 1988) has pointed out that recovery of exogenous spikes added during sample preparation does not necessarily reflect recovery of endogenous compounds in foods. Resolution of discrepancy between our wheat B6 values and those in earlier reports will await future studies measuring recovery of intrinsically labeled B6 vitamers (Gregory 1988).

A second conclusion from our data is that a significant fraction (average of 68%) of vitamin B6 in wheat is present as PNG. There is a paucity of reports documenting PNG content of whole wheat, and only one report dealing with PNG in wheat flour (Kabir et al 1983). Total B6 and PN content of wheat bran (≈60 and 23 nmol/g, respectively) is reported to be three-fold higher than in wheat or flour (Michaela and Lorenz 1976, Kabir et al 1983, Tadera et al 1986, Gregory and Sartain 1991). In two of these reports (Tadera et al 1986, Gregory and Sartain 1991), PNG, measured using HPLC, comprised a significant portion of total vitamin B6 (≈40%) (Gregory and Sartain 1991). These observations of PNG in wheat bran are consistent with our data that a significant portion of wheat B6 is PNG (Toepfer and Lehmann 1961, Polansky et al 1964, Toepfer and Polansky 1964, 1970, Toepfer et al 1972, Michaela and Lorenz 1976, Kabir et al 1983).

PNG has bioavailability in human subjects of ≈58% (Gregory et al 1991) compared to PN, so that the amount of available B6 in wheat will be significantly lower than the total amount if PNG were present in significant amounts. This could be potentially important for vitamin B6 nutritional status in individuals who habitually consume large quantities of unfortified whole wheat products, especially if their diet provides marginal amounts of B6 relative to the RDA. Food intake surveys have shown repeatedly that many Americans consume inadequate amounts of vitamin B6.

Glycosylated forms of vitamin B6 other than PNG occur in wheat, but at levels much lower than for PNG itself (Tadera et al 1988, Gregory and Sartain 1991). Our method's relative quickness and simplicity precluded our obtaining information in the wheats we analyzed about these other minor glycosylated forms of B6.

The source of variation in wheat PN and PNG reported here may be storage time and condition, seasonal variation, and geographic variation.

The variation in vitamin B6 content for flour vs. whole wheat confirms earlier reports (Schroeder 1971) showing that milling removes a significant portion (≈75%) of vitamin B6. This observation emphasizes that milling significantly decreases the nutrient density of vitamin B6 in wheats.

In conclusion, our results indicate that wheat contains variable amounts of vitamin B6 with significant amounts accounted for by PNG. Our observations about vitamin B6 in American vs. Canadian wheats, Colorado vs. other wheats, and hard vs. soft wheats emphasize the larger point that vitamin B6 content of wheats varies considerably between cultivars. Using water and metaphosphoric acid extraction, we have observed vitamin B6 content in wheats that are lower than in earlier reports using standard acid extraction procedures (AOAC). Resolution of this numerical disparity awaits a future study focused on comparison of extraction methods. Our primary interest and conclusion is that vitamin B6 concentration varies significantly within wheats, and that PNG accounts for a significant percentage of vitamin B6 in wheats.

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