# Thiamine and Folate in Potato: Targets for Increased Nutritional Value and Enhanced Disease Resistance

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## Abstract

The development of more nutritious potato varieties would *(1)* benefit those for which it is a diet staple, *(2)* help the potato industry cope with negative publicity associated with glycemic index and acrylamide issues, and *(3)* open up new economic opportunities for growers and processors. Thiamine and folate are essential micronutrients in the human diet. These water-soluble B vitamins are great targets for nutritional enhancement of potato because *(1)* thiamine and folate deficiencies are still common throughout the world, even in developed countries, and are responsible of various serious diseases, *(2)* the potato is relatively poor in thiamine and folate compared to rich sources such as beans and lentils, and *(3)* there is little loss of thiamine and folate during cooking and processing. In addition, there is increasing evidence that higher thiamine content correlates with increased resistance to biotic and abiotic stresses in plants. Unfortunately, the natural variation of thiamine and folate concentrations in potato has not been extensively studied. Therefore, the author measured thiamine and folate concentrations in various potato genotypes in order to evaluate the potential for increase of these vitamins in new varieties. Field-grown tubers from commonly grown varieties and advanced breeding lines contained between 410 and 871 ng thiamine per g fresh weight (FW). The higher ones would qualify as good or excellent sources of thiamine. Wild species grown in a greenhouse had even higher variation in thiamine content, ranging from 476 to 2104 ng per g FW. Primitive cultivars contained between 600 and 1264 ng thiamine per g FW, and had between 159 and 385 ng folate per g FW.

## Introduction

Potatoes with improved nutritional qualities offer a more appealing product for consumers and help offset negative publicity associated with glycemic index and acrylamide issues. In particular, there is an increased demand for specialty potatoes which is partially due to the higher demand from consumers for more colorful, tastier, and more nutritious potatoes, and partially due to the pro-active small-scale farmers who are trying to diversify their production and marketing to provide economic stability. To answer this demand, varieties such as Purple Pelisse, a dark purple flesh potato high in antioxidants selected by the Pacific Northwest Tri-State Breeding Program, was recently released. The identification of genotypes high in phytonutrients is the starting point to developing commercially available high-phytonutrient potato varieties. The potato possesses a tremendous genetic diversity that has only just begun to be utilized. Amongst the thousands of existing potato accessions, some will hopefully be rich in thiamine and folate, the vitamins currently being investigated.

Thiamine and folate are essential micronutrients in the human diet. Thiamine deficiency is still common throughout the world [1], even in the U.S. where cases of thiamine deficiency are reported every year, and are linked to various diseases such as heart failure and bladder dysfunction [2]. A deficiency of folate in the diet is associated with the increased risk of neural tube defects, strokes, cardiovascular diseases, anemia and some cancers. Unfortunately, current folate intake is suboptimal in most of the world's populations, even in developed countries [3]. Every year, ca. 500,000 newborns are affected by birth defects due to inappropriate prenatal folate intake by their mothers. Unfortunately, potato varieties currently consumed are relatively low in folate and thiamine. Concentrations in modern potato varieties are four to ten times lower than in sources such as beans or lentils (The USDA Nutrient Database SR20). Despite its relatively low thiamine and folate content, potato has two major advantages over other foods in its capability of being developed as a major source of these vitamins: *(1)* it is the third most consumed vegetable worldwide; and *(2)* when cooking or processing potatoes there is little loss of thiamine and folate. Thus, the introduction of new potato varieties high in thiamine and/or folate could significantly decrease the incidence of diet deficiencies.

In addition to its importance in nutrition, thiamine exhibits potential for increased disease resistance in crops. Thiamine has been shown to stimulate the plant's inherent defense mechanisms which lead to protection against bacterial, viral, and fungal diseases [4]. Plants sprayed with thiamine and then infected with pathogens either showed significantly decreased or no disease symptoms. Rice with lower thiamine content has been shown to be more susceptible to bacterial blight and blast [5]. These results suggest that cultivars with higher thiamine content could be more resistant to select pathogens.

The natural variation of thiamine and folate concentrations in potato has not been extensively studied. This project was focused on determining the range of thiamine and folate concentrations amongst potato genotypes from the Northwest breeding programs, some popular varieties, and a series of wild species and primitive cultivars. Here, the author reports on thiamine and folate concentrations in these various potato genotypes.

## Materials and Methods

**Potato Material.** During the 2009 season, tubers were harvested from 46 breeding lines or cultivars grown at the Hermiston Agricultural Research and Extension Center (HAREC). These included 16 varieties selected by the Pacific Northwest Tri-State Breeding Program, 20 advanced breeding lines from the Tri-State Breeding Program, and 10 commonly grown varieties. Tubers were harvested by hand early in the growing season (mid-June) and weighed 16 g on average. A small collection of wild potato species accessions were grown in a HAREC greenhouse in 2008 and were harvested mid-November under short-day conditions. Primitive cultivars were field-grown at Sturgeon Bay and were harvested mid-September. Tubers weighed 36 g on average. These tubers were a kind gift from Dr. John Bamberg. All tubers were frozen in liquid nitrogen at harvest or one day post-harvest.

**Thiamine Assay.** Thiamine concentrations were measured by using a microbiological assay employing *Lactobacillus viridescens* grown in thiamine deficient medium. Total thiamine was extracted by acid hydrolysis and enzymatic treatment with takadiastase. After extraction, the solution was adjusted to pH 6.5 and centrifuged. Assays were performed on 96-well microtiter plates. Plates were incubated at 30°C for ca. 18 h. Bacterial growth was measured at 650 nm on a BioTek Instrument EL 311 SX microplate autoreader and analyzed with the KCJr EIA application software. Thiamine concentrations were calculated by reference to a standard curve using known amounts of thiamine. Concentrations are the average of two independent measurements performed on at least two extracts from each of three independent biological samples.

**Folate Assay.** Folates were extracted by a tri-enzyme treatment (protease, amylase, and deconjugase) in 10 mL of extraction buffer (50 mM HEPES/50 mM CHES, pH 7.85, containing 2% (w/v) sodium ascorbate and 10 mM 2-mercaptoethanol, deoxygenated by flushing with nitrogen). After centrifugation for 10 min at 3000*g*, the supernatant was transferred to a new tube. The residue was re-suspended in 5 mL of extraction buffer and re-centrifuged for 10 min. The combined supernatants were adjusted to a 20-mL final volume with extraction buffer, flushed with nitrogen, frozen in liquid nitrogen, and stored at -80°C until analysis. *Lactobacillus rhamnosus* was used to determine total folate content of tri-enzyme-treated samples. Assays were performed on 96-well microtiter plates. Plates were incubated at 37 °C for 18 h. Bacterial growth was measured at 650 nm on a BioTek Instrument EL 311 SX microplate autoreader and analyzed with the KCJr EIA application software. Results were calculated by reference to a standard curve using 5-formyl-tetrahydrofolate. Concentrations are the average of two independent measurements performed on at least two extracts from each of three independent biological samples.

## Results and Discussion

Thiamine concentrations in tubers from field-grown varieties ranged between 410 and 871 ng thiamine $g^{-1}$ FW (ca. 2.2-fold variation) (Table I). Amongst all these genotypes, twelve would provide over 10% of the Recommended Daily Allowance (RDA) based on mid-size tuber servings (175 g) and would qualify as a “good source” of thiamine according to the USDA grading system. Previous reports have shown that thiamine retention during cooking is high, 95% and 100% for microwaved and boiled unpeeled potatoes, respectively [6]. Therefore we expect that values reported here on raw tubers would be about the same or slightly lower after cooking.

Wild species grown in a greenhouse showed a higher variation in thiamine content than established cultivars, ranging from 476 to 2104 ng $g^{-1}$ FW (Fig. 1). The two highest thiamine genotypes had more than double the amount of thiamine than that of Russet Burbank and could therefore be used as source of genes to introgress into domesticated cultivars for nutritional improvement.

We also determined thiamine and folate concentrations in a small set of primitive cultivars. Thiamine concentrations ranged between 600 and 1264 ng $g^{-1}$ FW (ca. 2.1-fold variation) (Fig. 2). Thiamine concentrations differed significantly between field and greenhouse grown Russet Burbank tubers as well as between field locations (Table I, Fig. 1 and 2). This could be due to differences in environmental conditions under which potato plants were grown and/or the stage of development at which tubers were harvested. We observed a significant increase in thiamine concentrations during tuber enlargement (data not shown). Folate concentrations ranged between 159 and 385 ng $g^{-1}$ FW (Fig. 3), the highest genotype containing 2.4-fold more folate than Russet Burbank. A mid-size tuber serving of the highest folate genotype would provide ca. 17% of the RDA and would qualify as a “good source” of folate. The highest folate genotype was also the highest thiamine genotype.

## Conclusions

Thiamine and folate concentrations varied substantially amongst potato genotypes grown under the same environmental conditions (one location/one year). In particular, wild species and primitive cultivars showed the greatest range of vitamin concentrations. Potato genotypes identified to have high levels of thiamine and folate could provide genes to introgress into modern cultivars for nutritional enhancement and possibly increased disease resistance.

## References


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**Table I:** Thiamine concentrations in tubers from 46 potato varieties field grown at HAREC. The Recommended Daily Allowance (RDA) for a healthy male adult is 1.2 mg per day. The percentage of the RDA provided by a raw potato serving is indicated in the last two columns. Food servings which provide over 10% of the RDA are qualified as a "good source"; those which provide over 20% of the RDA are qualified as an "excellent source" according to the USDA grading system. SE, Standard Error.

| | ng/g FW | SE | %RDA (175 g serving) | %RDA (300 g serving) |
|---|---|---|---|---|
| A00286-3Y | 410 | 23 | 6 | 10 |
| Umatilla Russet | 428 | 18 | 6 | 11 |
| Yukon Gold | 432 | 27 | 6 | 11 |
| Wallowa Russet | 460 | 17 | 7 | 12 |
| Shepody | 465 | 16 | 7 | 12 |
| Abnaki | 475 | 21 | 7 | 12 |
| Klamath Russet | 483 | 17 | 7 | 12 |
| PA96RR1-193 | 496 | 37 | 7 | 12 |
| Ranger Russet | 508 | 66 | 7 | 13 |
| R. Burbank | 510 | 37 | 7 | 13 |
| POR01PG22-1 | 514 | 32 | 7 | 13 |
| Russet Norkotah | 515 | 40 | 8 | 13 |
| POR03PG23-1 | 523 | 27 | 8 | 13 |
| Purple Majesty | 538 | 33 | 8 | 13 |
| A96814-65LB | 543 | 17 | 8 | 14 |
| Achirana | 545 | 17 | 8 | 14 |
| Dark Red Norland | 564 | 21 | 8 | 14 |
| AO96160-3 | 577 | 26 | 8 | 14 |
| Modoc | 578 | 19 | 8 | 14 |
| Alturas | 583 | 20 | 9 | 15 |
| Mazama | 589 | 23 | 9 | 15 |
| POR02PG26-5 | 590 | 19 | 9 | 15 |
| Clearwater Russet | 599 | 26 | 9 | 15 |
| Classic Russet | 602 | 37 | 9 | 15 |
| Defender | 607 | 24 | 9 | 15 |
| All Blue | 619 | 45 | 9 | 15 |
| POR03PG80-2 | 620 | 26 | 9 | 16 |
| A99331-2RY | 624 | 21 | 9 | 16 |
| POR01PG20-12 | 635 | 38 | 9 | 16 |
| POR01PG10-1 | 636 | 37 | 9 | 16 |
| Highland Russet | 640 | 26 | 9 | 16 |
| POR01PG45-5 | 642 | 37 | 9 | 16 |
| Gem Russet | 643 | 30 | 9 | 16 |
| AOR00681-15 | 644 | 21 | 9 | 16 |
| COO86107-1 | 656 | 39 | 10 | 16 |
| POR02PG37-2 | 658 | 45 | 10 | 16 |
| POR01PG1-6 | 660 | 52 | 10 | 17 |
| A97066-42LB | 663 | 19 | 10 | 17 |
| OR00068-11 | 669 | 27 | 10 | 17 |
| Purple Pelisse | 680 | 42 | 10 | 17 |
| Blazer Russet | 688 | 27 | 10 | 17 |
| Red Sunset | 732 | 60 | 11 | 18 |
| Red LaSoda | 754 | 29 | 11 | 19 |
| GemStar Russet | 840 | 32 | 12 | 21 |
| Premier Russet | 856 | 36 | 12 | 21 |
| AOA95155-7 | 871 | 35 | 13 | 22 |

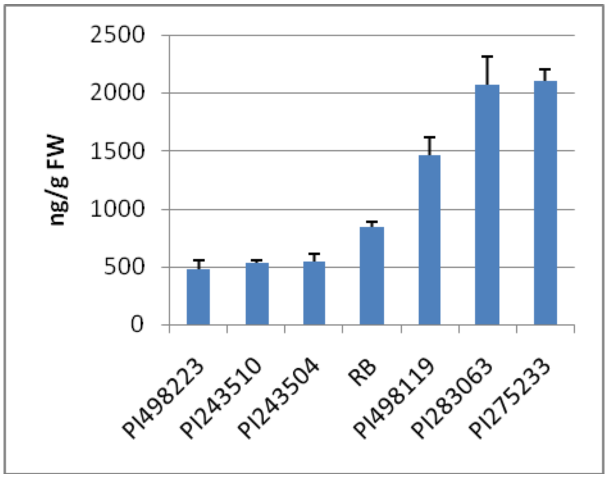


**Figure 1:** Thiamine concentrations in tubers of six wild potato species. All plants were grown in a greenhouse in 2008 at HAREC. RB, Russet Burbank; *Solanum bulbocastanum*: PI498223, PI243510, PI243504; *S. circaeifolium*: PI498119; *S. cardiophyllum*: PI283063; *S. pinnatisectum*: PI275233.

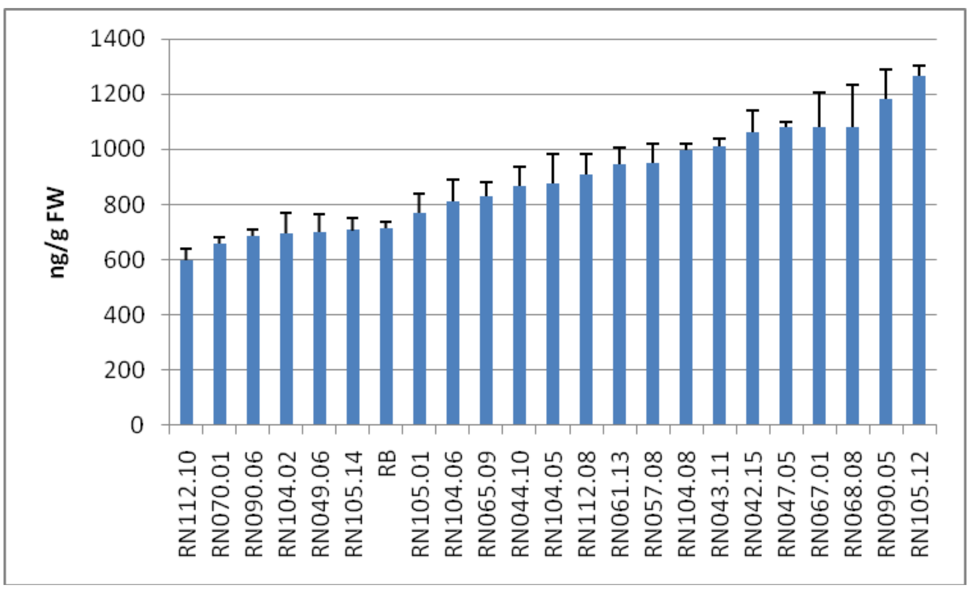


**Figure 2:** Thiamine concentrations in tubers of primitive cultivars. All plants were field grown in 2009 at Sturgeon Bay, WI. RB, Russet Burbank.

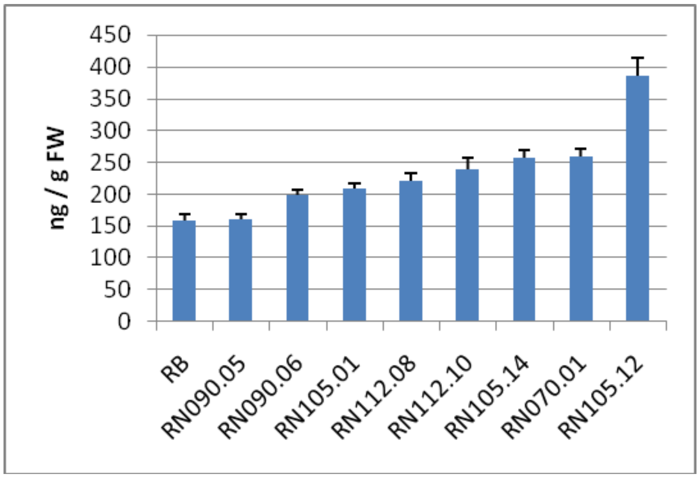


**Figure 3:** Folate concentrations in tubers of primitive cultivars. All plants were field grown in 2009 at Sturgeon Bay, WI. RB, Russet Burbank.