

# Carotenoids and Folate Vitamers in Indigenous Plants Consumed by Northern Plains Indians

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## Abstract

Epidemiological research strongly supports increased consumption of carotenoid- and folate-rich plant foods to reduce the risk of several chronic diseases. The folate DRI is 400µg (IOM, 1998), with evidence suggesting roles for specific vitamers. No recommended intake for individual carotenoids exists but some can be converted to vitamin A (IOM, 2000). Consumption of wild plants, an important source of these nutrients but where data are limited, has declined among American Indians. Eight wild plants historically consumed by Northern Plains Indians were analyzed separately for carotenoids and folate using HPLC; MS detection for folates enabled quantitation of individual vitamers: total carotenoids (mg/100g) were highest in rose hips (11.7), wild plums (3.2), raw and cooked lambsquarters (4.8, 8.5, respectively) and blanched stinging nettles (5.4). Cattail shoots, wild raspberries and chokecherries contained <5 mg/100g total edible plant. β-carotene, lutein, and zeaxanthin were the predominant carotenoids; lycopene was present only in rose hips (6.8 mg/100g). Significant folate (total >20 µg/100g) was found in cattail shoots and stinging nettles, and was highest in lambsquarters (>1). The major vitamer was 5-H<sub>3</sub>C-H<sub>4</sub>folate. Formylfolates (10-HCOfolate, 5-HCO-H<sub>4</sub>folate) were high (>30 µg/100g total) only in lambsquarters. With many tribes pursuing a return to traditional foods, additional analyses of indigenous wild plants are warranted.

## Introduction

The traditional diet of Native Americans has changed with dramatically decreased consumption of nutrient-dense harvested plants (Byers, 1996, Lytle et al., 2002). The Strong Heart Dietary Studies - focused on CVD risks, showed median dietary intakes of folate and vitamin A were lower than recommended among adult tribal members in North and South Dakota compared to median intakes among NHNES survey respondents (Stang et al., 2005). Survey results show Native Americans regard traditional foods such as foraged plants as health-promoting (Powers and Powers, 1990) but they are usually consumed only at special ceremonies and celebratory events (Zephier et al, 1997; deGonzague et al, 1999; Woolf et al, 1999). In a 1999 study of Ojibwe Indians, however, foraged plants were shown to be good sources of phytonutrients (deGonzague et al., 1999). In general, information is extremely limited on the nutrient contents of traditional Native American plant foods, especially among tribes in the Northern Plains (Woolf et al, 1999). Development of datasets that support reintroduction or increase in consumption of healthful, foraged plants is the objective of this research; data are available to the collaborating tribes and through USDA's website: [www.ars.usda.gov/nutrientdata](http://www.ars.usda.gov/nutrientdata).



**Figure 1. Foraged plants** (left to right , top to bottom) **rose hips, prairie turnips, lambsquarters, wild raspberries, chokecherries, cattail shoots, wild plums, stinging nettles**

## Methods

### Sampling

Source of commonly consumed wild plants from Native-owned land United Tribes Technical College (UTTC) and Tribal leaders and elders

- Turtle Mountain Band of Chippewa, (Belcourt, ND)
- Three Affiliate Tribes of Ft. Berthold, ND (Mandan, Hidatsa, Arikara)
- Standing Rock Sioux reservation, ND

Eight traditional plant foods collected (in a culturally-respectful manner) at three reservations in May/June of 2005 (Figs. 1, 2).

- Chokecherries (*Prunus virginiana*)
- Prairie turnips (*Rosa arkansana*)
- Rosehips (*Rosa arvensis*)
- Stinging nettle (*Urtica dioica* var *lyallii*)
- Lambsquarters (*Chenopodium album*)
- Wild raspberries (*Rubus stigosus* Michx.)
- Wild plums (*Prunus americana* Marsh.)
- Cattail shoots (*Typha* spp. L.)

Plants shipped refrigerated over-night delivery to Virginia Tech's Food Analysis Laboratory Control Center (FALCC) for processing.

- Samples either raw or cooked using guidance from tribal elders (e.g., steamed, blanched)
- Identified by location (e.g., reservation)
- Divided into samples for comprehensive nutrient analysis

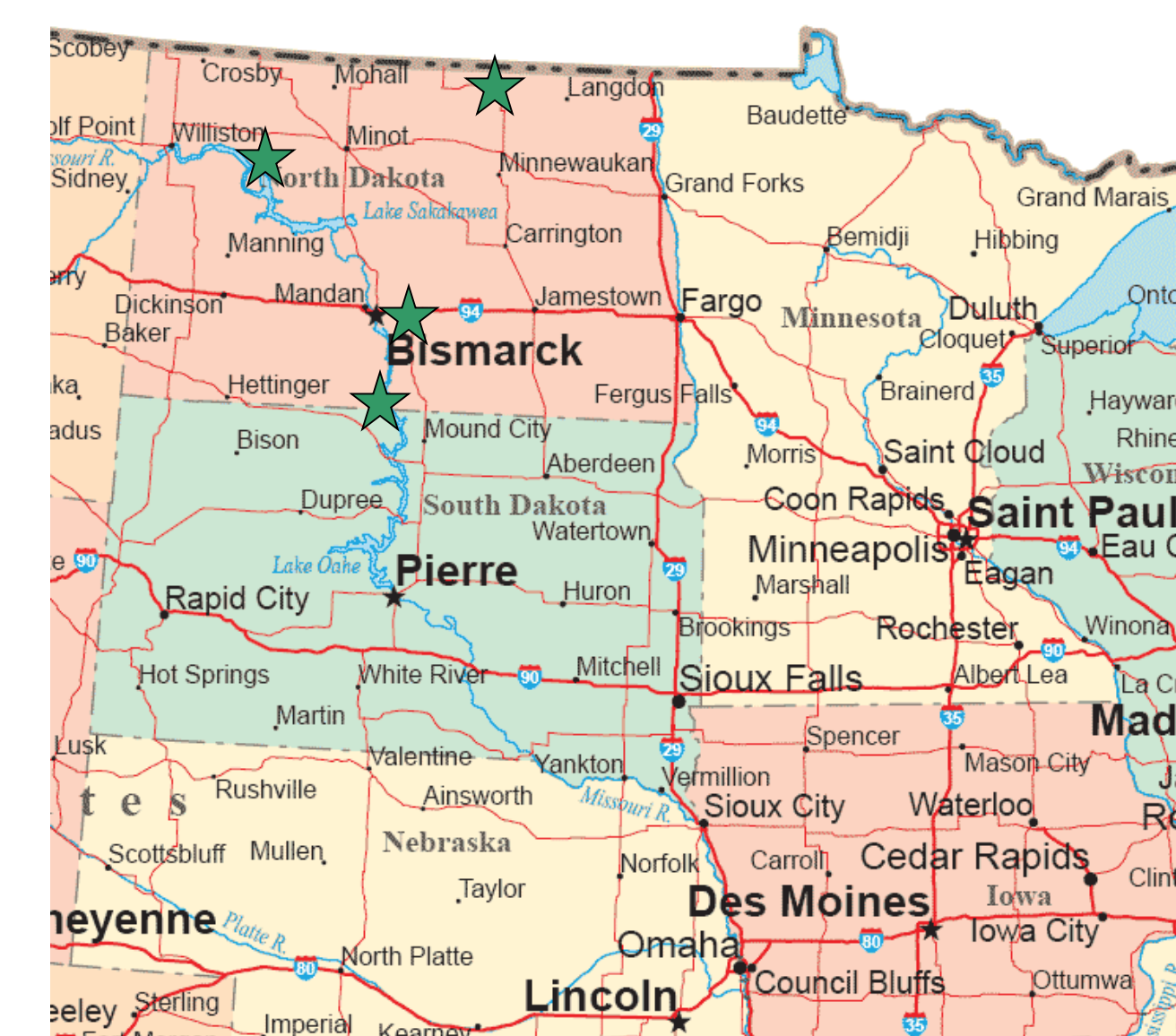
### Nutrient Analysis

#### Carotenoids (commercial lab)

- Wrolstad et al., Chromatographic Techniques for Carotenoid Separation, in Current Protocols in Analytical Chemistry (2001), sections F2.3.5 and F2.3.8.
- #### Folate (FALCC)
- Folate vitamers after trienzyme extraction of samples analyzed by LC-MS with external calibration for quantitation as previously reported:
  - Phillips, K.M., Ruggio, D.M., Ashraf-Khorassani, M., Haytowitz, D.B. (2006), J. Agric. Food Chem., 54:9998-10002.

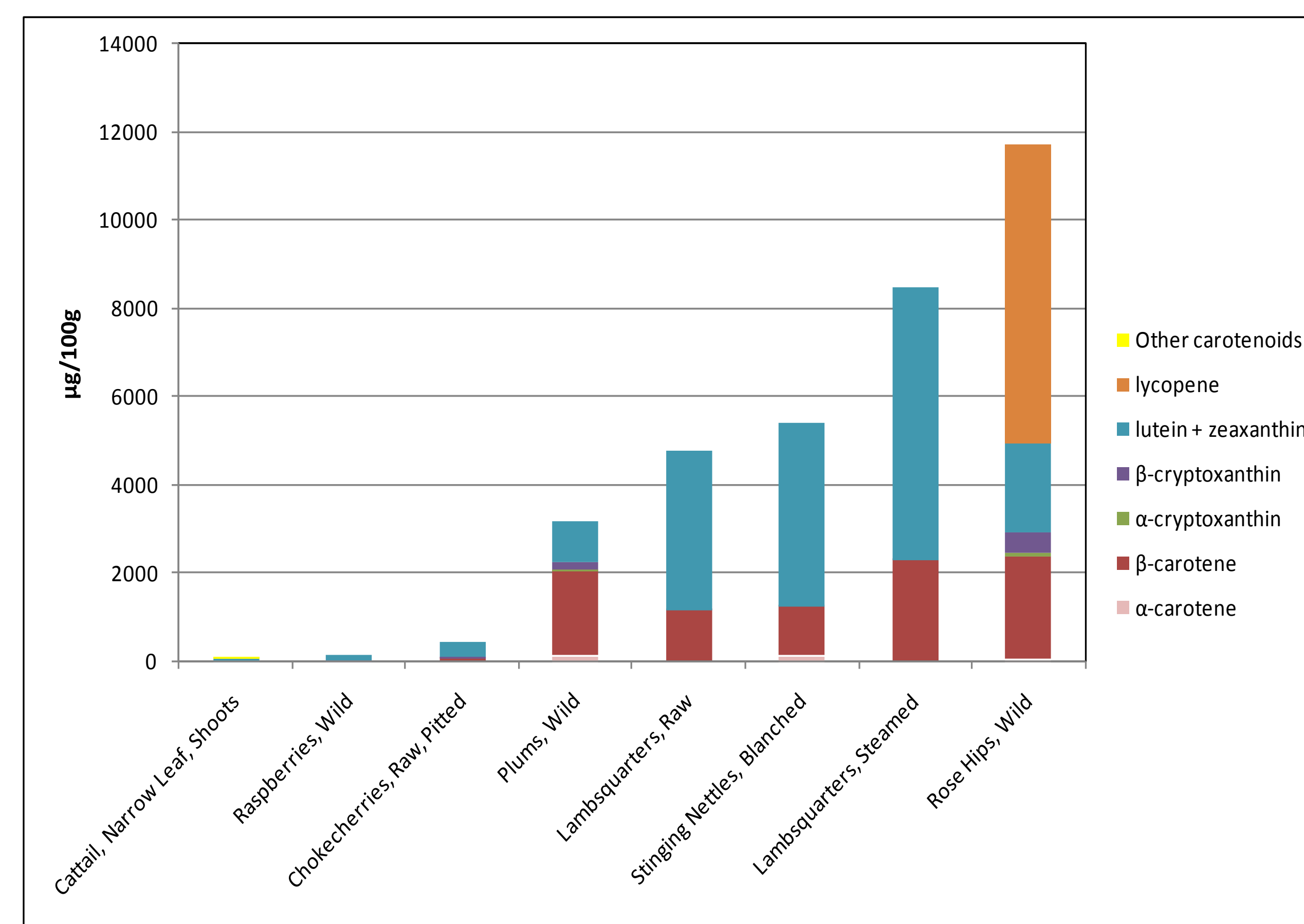
#### Analytical Quality Control

- Control materials run with all samples:
- For carotenoids - National Institute of Standards and Technology (NIST) reference materials SRM 2383 Baby Food (NIST, 2011)
  - For folates - BCR 485 Freeze Dried Mixed Vegetables from the Institute for Reference Materials and Measurements (IRMM), Belgium (Finglas, et al., 1998)
  - Other in-house control materials developed at Virginia Tech also used



**Figure 2. Sites of plant harvests: North Dakota reservations**

**Figure 3. Distribution of Carotenoids (µg/100g)**



**Table 1. Carotenoids Levels (mg/100g; mean (n, RSD))**

Food	α-Carotene	β-Carotene	α-Crypto-xanthin	β-Crypto-xanthin	Lutein + Zeaxanthin	Lycopene	Other <sup>a</sup> Carotenoids	Total
Cattail, Narrow Leaf, Shoots	nd	0.01	nd	nd	0.076	nd	46.3	0.13
Raspberries, Wild	0.002	0.01	nd	0.031	0.130	nd	nd	0.18
Chokecherries, Raw, Pitted	0.002	0.09	nd	0.019	0.347	nd	nd	0.46
Plums, Wild	0.140	1.93	0.030	0.187	0.920	nd	nd	3.18
Lambsquarters, Raw	nd	1.17	nd	0.0	3.616	nd	nd	4.79
Stinging Nettles, Blanched	0.114	1.15	nd	0.0	4.178	nd	nd	5.44
Lambsquarters, Steamed	nd	2.33	nd	0.0	6.162	nd	nd	8.49
Rose Hips, Wild	0.031	2.35	0.084	0.483	2.001	6.800	nd	11.75
NIST2383 Baby Food	0.090 (4, 5.8%)	0.309 (4, 2.7%)	0.150 (4, 2.3%)	0.148 (4, 23.6%)	0.662 (4, 23.1%)	0.084 (4, 8.1%)	nd	0.441 (9.0%) <sup>d</sup>
Certified <sup>b</sup>	0.067 - 0.099	0.249 - 0.375	Not assayed	0.107 - 0.169	0.155 - 0.249	0.550 - 0.850 <sup>c</sup>	nd	

<sup>a</sup>Unidentified. <sup>b</sup>Certified value, range. <sup>c</sup>Reference value. nd = not detected (< .002). <sup>d</sup>The inter-assay RSD for the carotenoid reference material (CRM) (NIST2383 Babyfood) was 9.0% for total carotenoids (range 2-24%). The RSDs for the CRMs provide an estimate of the analytical uncertainty around values shown for each food.

**Table 2. Levels of Folate Vitamers (µg/100g; mean (n, RSD))**

Food	5-H <sub>3</sub> C-H <sub>4</sub> folate	10-HCO folate	5-HCO-H <sub>4</sub> folate	Sum of folates <sup>a</sup>	Serving size (g)	%RDA <sup>b</sup> per serving
Cattail Narrow Leaf, Shoots	1.14	6	3.25	9.78	90	2.2
Stinging Nettles, Blanched	10.57	7.81	1.83	19.2	20	1.0
Prairie Turnips, Plains, Raw	13.72	0.55	1.28	14.9	130	4.8
Lambsquarters, Raw	46.8 (2, 3%)	42.9 (2, 13%)	12.2 (2, 14%)	96.8	30	7.3
Raspberries, Wild	7.32	0.18	1.47	8.58	61.5	1.3
Chokecherries, Raw, Pitted	0.86	0.27	2.28	3.21	75	0.6
Rose Hips, Wild	6.47	0.35	0.68	7.18	48	0.9
Plums, Wild	0.53	0.34	0.4	< 2	132	0.4
BCR485 freeze-dried mixed vegetables <sup>c</sup>	239 <sup>d</sup> (43, 6.3%)	<1.00 (12, n/a)	3.63 (11, 13.5%)	234 <sup>e</sup> (8, 7.1%) <sup>f</sup>		n/a

<sup>a</sup>As folic acid equivalent. <sup>b</sup>RDA = 400 µg/day. <sup>c</sup>Values on dry weight basis. <sup>d</sup>Information value by HPLC: (mean ± SD) = 172 - 256. <sup>e</sup>Certified total folate by microbiological assay (mean ± SD) = 287 - 343. <sup>f</sup>The RSDs for the folate CRM, BCR485 Lyophilized Mixed Vegetables, was 7.1% (range 6-13%), for the sum of folates. The RSDs for the CRMs provide an estimate of the analytical uncertainty around values shown for each food.

## Results and Conclusions

Total carotenoids (mg/100g; Fig. 3, Table 1) were highest in rose hips (11.75), wild plums (3.18), raw and cooked lambsquarters (4.79, 8.49, respectively) and blanched stinging nettles (5.42). The β-carotene content of rose hips was similar to that of baked sweet potatoes (11.5 mg/100g) (USDA, 2010). Cattail shoots, wild raspberries and chokecherries contained <5 mg carotenoids/100g total edible plant. Beta-carotene, lutein, and zeaxanthin were the predominant carotenoids; lycopene was present only in rose hips (6.8 mg/100g), providing over half of the total carotenoids.

Lambsquarters had the highest folate content but could still not be considered a good source (96.8 µg/100g, 7.3% RDA per serving, Table 2). Cattail shoots, prairie turnips, and stinging nettles contained low levels of folate (1-5% RDA/serving), while the remaining foods contained <10 µg/100g and ≤1% RDA/serving. The major vitamer was 5-H<sub>3</sub>C-H<sub>4</sub>folate, except in cattail shoots where formyl folates predominated. Lambsquarters contained a significant concentration of formyl folates, with 42.9 µg/100g as 10-HCOfolate and 12.2 µg/100g as 5-HCO-H<sub>4</sub>folate. The folate content of lambsquarters is similar to that of cooked turnip greens (8.9% of RDA per 30-g serving) (USDA, 2010).

Consumption of Plains wild plants may add important vitamins to the diet of Native tribes of the region. Of the plants examined, lambsquarters were the best source of folate and rose hips the best source of carotenoids. Many of these plants are dried and preserved for year round consumption and should be sampled and analyzed in future studies. With many tribes pursuing a return to traditional foods, additional analyses of indigenous wild plants are warranted.

## References

- Byers T. (1996). Nutrition and cancer among American Indians and Alaska Natives. Cancer 78: 1612-1616.
- deGonzague B, Receveur O, Wedli D, Kuhnlein HV. (1999). Dietary intake and body mass index of adults in 2 Ojibwe communities. JADA 99(6):710-716.
- Finglas, P. M., Scott, K. J., Witthöft, C. M., van den Berg, H., & de Froidmont-Görtz, I. (1998). The certification of the mass fractions of vitamins in four reference materials: wholemeal flour (CRM 121), milk powder (CRM 421), lyophilized mixed vegetables (CRM 485), and lyophilized pigs liver (CRM 487). EUR Report 18320. Luxembourg: Office for Official Publications of the European Communities.
- Institute of Medicine (IOM). (2000). Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. National Academy Press, Washington DC.
- Institute of Medicine (IOM). (1998). Dietary Reference Intakes (DRI) for Thiamin, Riboflavin, Niacin, Vitamin B<sub>6</sub>, Folate, Vitamin B<sub>12</sub>, Pantothenic Acid, Biotin, and Choline. National Academy Press, Washington DC.
- Lytle L, Dixon L, Cunningham-Sabo L, Evans M, Gittelsohn J, Hurley J, Snyder P, Stevens J, Weber J, Antiker J, Heller K, Story M. (2002). Dietary intakes of Native American children: findings from the Pathways Feasibility Study. JADA 102: 555-559.
- National Institute of Standards and Technology (NIST). <http://www.nist.gov/srm/>, accessed April 4, 2011.
- Phillips KM, Ruggio DM, Ashraf-Khorassani M, Haytowitz DB. (2006). Difference in folate content of green and red sweet peppers (Capsicum annum) determined by liquid chromatography-Mass Spectrometry. J Agric Food Chem 54:9998-10002.
- Powers WK, Powers MN. Sacred Foods of the Lakota. Kendall Park, NJ: Lakota Books, 1990. 61 pp.
- Stang J, Zephier EM, Story M, Himes JH, Yeh JL, Welty T, Howard BV. (2005). Dietary Intakes of Nutrients Thought to Modify Cardiovascular Risk from Three Groups of American Indians: The Strong Heart Dietary Study, Phase II. JADA 105(12):1895-1903.
- USDA, Agricultural Research Service, Beltsville Human Nutrition Research Center, Nutrient Data Lab. USDA National Nutrient Database for Standard Reference (SR). USDA: Beltsville, MD. 2010. USDA website: <http://www.ars.usda.gov/nutrientdata>.
- Woolf N, Conti KM, Johnson C, Martinez V, McCloud J, Zephier EM. (1999). Northern Plains Indian Food Practices, Customs and Holidays. Ethnic and Regional Food Practices - a Series. Chicago, IL: American Dietetics Association.
- Zephier EM, Ballew C, Mokdad A, Mendlein J, Smith C, Yeh JL, Lee E, Welty TK, Howard B. (1997). Intake of nutrients related to cardiovascular disease risk among three groups of American Indians: The Strong Heart Dietary Study. Prev Med 26: 508-51.

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