# Fagopyritols, D-*chiro*-Inositol, and Other Soluble Carbohydrates in Buckwheat Seed Milling Fractions

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Fagopyritols are mono-, di-, and trigalactosyl derivatives of D-*chiro*-inositol that accumulate in seeds of common buckwheat (*Fagopyrum esculentum* Moench) and may be important for seed maturation and as a dietary supplement. Fagopyritols and other soluble carbohydrates were assayed in mature groats and 11 milling fractions of common buckwheat seed. Because fagopyritols are in embryo and aleurone tissues, differences in fagopyritol concentrations reflect varying proportions of these tissues in each milling fraction. Bran milling fractions contained 6.4 g of total soluble carbohydrates per 100 g of dry weight, 55% of which was sucrose and 40% fagopyritols. Flour milling fractions had reduced fagopyritol concentration [0.7 g/100 g of dry weight total fagopyritols in the dark (Supreme) flour and 0.3 g/100 g in the light (Fancy) flours]. Fagopyritol B1 was 70% of total fagopyritols in all milling fractions. Fagopyritols were 40% of total soluble carbohydrates in groats of two cultivars of common buckwheat but 21% in groats of tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn.], probably a reflection of environment and genetics. A rhamnoglucoside present in tartary buckwheat was not detected in common buckwheat.

**Keywords:** Fagopyrum esculentum; Fagopyrum tataricum; buckwheat seed milling fraction composition; fagopyritol; D-chiro-inositol

## INTRODUCTION

Fagopyritols are galactosyl derivatives of D-chiroinositol that accumulate in seeds of some species. Seeds of common buckwheat (Fagopyrum esculentum Moench) accumulate six fagopyritols (Horbowicz et al., 1998). Fagopyritol B1 [O- $\alpha$ -D-galactopyranosyl-( $1 \rightarrow 2$ )-D-*chiro*inositol (Horbowicz et al., 1998; Szczeciński et al., 1998)] is the major fagopyritol accumulating in buckwheat embryos (axis and cotyledons) and aleurone layer cells of the endosperm 12-20 days after pollination. Fagopyritol B1 has been identified in seeds of soybean (Schweizer and Horman, 1981), lupin, lentil, and chickpea (Quemener and Brillouet, 1983), and jojoba bean (Ogawa et al., 1997). Chemical synthesis of fagopyritol B1 has been reported (Kornienko et al., 1998). Fagopyritol B2 [O- $\alpha$ -D-galactopyranosyl-( $1 \rightarrow 6$ )-O- $\alpha$ -D-galactopyranosyl- $(1 \rightarrow 2)$ -D-*chiro*-inositol] is present in buckwheat seeds in lesser quantities (Horbowicz et al., 1998) and has also been identified in seed balls of sugar beet (Shiomi et al., 1988). Fagopyritol B3 [O-α-D-galactopyranosyl- $(1\rightarrow 6)$ -O- $\alpha$ -D-galactopyranosyl- $(1\rightarrow 6)$ -O- $\alpha$ -D-galactopyranosyl- $(1 \rightarrow 2)$ -D-*chiro*-inositol] is present in small amounts in buckwheat seeds (K. J. Steadman, M. S. Burgoon, and R. L. Obendorf, Cornell University, unpublished results). Fagopyritol A1, fagopyritol A2, and fagopyritol A3 are positional isomers of fagopyritol B1,

fagopyritol B2, and fagopyritol B3, respectively (Horbowicz et al., 1998; Steadman et al., Cornell University, unpublished results).

Fagopyritols are structurally similar to a galactosamine D-chiro-inositol (Berlin et al., 1990), which is related to a pH 2.0 (type P) putative insulin mediator (Larner et al., 1988). Subjects with non-insulin-dependent diabetes mellitus (NIDDM, type II diabetes) appear to have abnormal metabolism of D-chiro-inositol (Kennington et al., 1990; Ostlund et al., 1993), a deficiency in *D-chiro*-inositol biosynthesis (Pak et al., 1998), and reduced levels of a pH 2.0 (type P) putative insulin mediator (Asplin et al., 1993; Shashkin et al., 1997). Dietary treatment with D-chiro-inositol may be effective in reducing symptoms of NIDDM (Ortmeyer et al., 1995; Hansen and Ortmeyer, 1996). Consumption of tartary buckwheat as flour or biscuits has been demonstrated to have hypoglycemic effects in diabetic patients (Lu et al., 1992; Wang et al., 1992), but the active components were not identified. The potential for the consumption of buckwheat, and particularly the consumption of fagopyritols, to reduce symptoms of NIDDM is of considerable interest.

Sucrose accumulates in buckwheat embryo and aleurone tissues. Only small amounts of raffinose and stachyose are present in hypocotyl tissues of the mature embryo (Horbowicz and Obendorf, 1994; Hobowicz et al., 1998).

Fagopyritols are concentrated in embryo and aleurone tissues (Horbowicz et al., 1998). Milling fractions that contain large proportions of these tissues would be expected to contain high concentrations of fagopyritols. Herein we report the fagopyritol composition of buck-

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wheat seeds and milling fractions that vary in the proportional composition of embryo, endosperm, and maternal (seed coat, hull) tissues. Fagopyritol and other soluble carbohydrate composition of two cultivars of common buckwheat and one of tartary buckwheat are reported.

### EXPERIMENTAL PROCEDURES

Materials. Common buckwheat (Fagopyrum esculentum Moench cv. Manor; Polygonaceae) groats and 11 milling fractions were provided by Minn-Dak Growers, Ltd. (Grand Forks, ND) during 1996 and 1997. The dicotyledonous buckwheat embryo traverses the starchy endosperm in a triangular seed enclosed by pericarp (hull) of the mature achene (fruit). Cotyledons of the embryo wrap around the endosperm adjacent to the seed coat. Buckwheat seed milling fractions (differing in proportions of embryo, seed coat, hull, endosperm, and particle size) were produced either by milling the intact achene (6/96, 10/96, 2/97) or by removing the hull through impact dehulling and then milling the resulting groats (4/97, 6/97, 9/97). In both cases milled achene or milled groat tissues were separated into Fancy flour and bran fractions. The aleurone layer and the outer of two cotyledons adhering to the seed coat separated with the bran. Larger fragments of cotyledons and the axis in the central endosperm separated with the bran also, but some soft embryo tissue was pulverized and separated with Fancy flour (rich in starch).

When intact achenes were milled, small pieces of hulls separated with the bran (batches 1 and 2, 10/96). Bran additionally processed to remove hull fragments was labeled bran (hull removed (2/97). Grits (10/96) included large, hard chunks of endosperm that resisted granulation.

Whole groats (4/97) were produced by sizing achenes, dehulling the achenes by impact dehulling (groats were forced out of the hulls by centrifugal force), air separation of the hulls (larger intact pieces) from the groats, and finish cleaning of the groats. Subsequent milling of the groats used corrugated roller mills for the first break followed by sifting to separate coarse particles from fine particles. Smooth rollers reduced coarse particles into bran and Fancy flour. Particle size of the bran (6/97) milling fraction was intermediate between coarse and finely ground bran (9/97). Supreme flour was derived from byproducts of the dehulling process, essentially a whole groat flour with some hull added. Whole groats, bran (Farinetta), and grits were further ground in a Wiley mill to pass a 40-mesh screen before analysis. Fancy flour and Supreme flour were used as received (100% passed through 40-mesh screen).

Soluble carbohydrate concentrations were determined for 11 milling fractions of common buckwheat cv. Manor, 5 of which were derived from milling intact achenes and 6 from milling groats after the removal of hulls and whole groats (lot 1). Achenes of one additional seed lot of common buckwheat cv. Manor (lot 2), two seed lots of common buckwheat cv. Mancan (lots 1 and 2), and one seed lot of tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn.] were dehulled by hand before analysis.

Carbohydrate standards, phenyl  $\alpha$ -D-glucoside (internal standard), quercetin, and trimethylsilylimidazole (TMSI) were purchased from Sigma Chemical Co. (St. Louis, MO). Pyridine was purchased from Regis Technologies (Morton Grove, IL). Fagopyritol standards were purified from buckwheat (Horbowicz et al., 1998). Galactinol and D-*chiro*-inositol standards were gifts.

**Moisture.** The moisture concentration of three replicates for each milling fraction was determined by drying at 98 °C in a vacuum oven under 700 mmHg vacuum for 6 h according to AOAC method 925.09 (AOAC, 1997).

**Extraction of Soluble Carbohydrates.** Three replicate 2.5-g samples of bran or groats and 5-g samples of flour or grits were blended for 2 min using a Brinkmann PT 2000 homogenizer with 20 mL of ethanol/water (1:1, v/v) containing 10 mg (bran), 5 mg (groats), or 2 mg (flour, grits) of phenyl  $\alpha$ -D-glucoside as internal standard. The mixture was centri-

fuged for 10 min at 43500g, the supernatant was removed, and the residue was re-extracted two times with 10 mL of ethanol/water (1:1, v/v) for 2 min and recentrifuged. An aliquot of the combined extracts was filtered through a 10000 MW cutoff filter (Pall Filtron, Northborough, MA), transferred to silylation vials, and evaporated to dryness in a stream of nitrogen gas.

Analysis of Soluble Carbohydrates. Extract residues were kept overnight in a desiccator over phosphorus pentaoxide to remove traces of water. Dry residues were derivatized with a silylation mixture (TMSI/pyridine, 1:1, v/v) in silylation vials at 70 °C for 30 min, cooled, and analyzed by highresolution gas chromatography (Horbowicz and Obendorf, 1994) using a Hewlett-Packard 5890 series II gas chromatograph equipped with a split-mode injection port (1:50) and flame ionization detector. Soluble carbohydrates were separated on a DB-1 capillary column (15 m  $\times$  0.25 mm i.d., 0.10  $\mu$ m film thickness; J&W Scientific, Folsom, CA) operated with an initial temperature of 150 °C, programmed to 200 °C at 3  $\,$ °C/min and then to 325 °C at 7 °C/min, and held at 325 °C for 20 min. The injection port was operated at 335 °C and the detector at 350 °C. The carrier gas was helium at 3.0 mL/min (measured at 30 °C).

Soluble carbohydrates were identified by GC retention times identical to those for standards, by GC-MS, and by analysis of hydrolysis products. Soluble carbohydrates were quantified from standard curves; the ratios of the area of signals for each known compound to the area of the signal for phenyl  $\alpha$ -D-glucoside, the internal standard, were plotted against known amounts of each compound (Horbowicz and Obendorf, 1994). Amounts of components for which no standard was available were estimated by comparison with the nearest standard. Amounts below the level of detection are presented as zero. Total soluble carbohydrates are reported as the sum of the individually quantified soluble carbohydrates.

### RESULTS

Buckwheat milling fractions derived from groats, that is, milled after removal of the hull (pericarp) of the achene (mature dry fruit), contained higher (p < 0.05) concentrations of fagopyritols than fractions originating from the milling of intact achenes. This was particularly apparent for the bran milling fraction; bran that included hull contained fagopyritols at only 0.9 g/100 g of dry weight (Table 1), whereas bran milled from groats contained 2.6 g/100 g of dry weight (Table 2; average of three fractions). Removal of hull fragments from milled achene bran significantly increased (220–250%) the concentration of total fagopyritols (bran, hull removed, 2/97) (Table 1), but the values were still significantly lower (p < 0.05) than those for the bran fractions milled from groats (Table 2).

Fancy flour milling fractions were low in fagopyritols, containing <0.4 g/100 g of dry weight (Tables 1 and 2). Grits, composed of hard chunks of central endosperm that resist granulation, were low in fagopyritols and other soluble carbohydrates. Supreme flour (9/97), consisting of dehulled groat pieces plus hull fragments, had a higher (p < 0.05) concentration of fagopyritols than Fancy flour (Table 2).

In all milling fractions, sucrose was 55% of the soluble carbohydrates and total fagopyritols were  $\sim$ 40%. Fagopyritol B1 was 70% of total fagopyritols, with a maximum concentration of 2 g/100 g of dry weight.

Mature groats of common buckwheat cv. Manor (Tables 2 and 3) contained a significantly higher (p < 0.05) concentration of total soluble carbohydrates and cyclitols than cv. Mancan (Table 3), but sucrose was 44–60% and fagopyritols were 35–43% of total soluble carbohydrates for both cultivars. The concentration of

# Table 1. Fagopyritol and Other Soluble Carbohydrate Composition of Buckwheat (Cv. Manor) Milling Fractions from Whole Achenes<sup>a</sup>

	buckwheat milling fractions from achenes (mg/100 g of dry wt)					
soluble carbohydrate	Fancy flour (6/96)	grits (10/96)	bran (batch 1, 10/96)	bran (batch 2, 10/96)	bran (hull removed, 2/97)	
D-chiro-inositol fagopyritol A1 fagopyritol A2 fagopyritol B3 fagopyritol B3 <i>myo</i> -inositol galactinol digalactosyl- <i>myo</i> -inositol sucrose total soluble carbohydrates total fagopyritols total D-chiro-inositol free D-chiro-inositol	$\begin{array}{c} 17.0\pm0.4\\ 34.5\pm3.3\\ 16.7\pm1.0\\ 11.8\pm0.7\\ 205.4\pm11.8\\ 23.2\pm3.0\\ \mathrm{tr}\\ 6.1\pm0.9\\ 4.3\pm0.4\\ 5.4\pm0.9\\ 392.6\pm35.2\\ 717.0\pm50.0\\ 291.6\pm16.3\\ 153.2\pm8.2\\ 17.0\pm0.4\\ \end{array}$	$\begin{array}{c} 6.9\pm 0.4\\ 12.4\pm 0.5\\ 9.2\pm 0.8\\ 11.1\pm 1.9\\ 78.9\pm 4.7\\ 16.7\pm 1.3\\ \mathrm{tr}\\ 3.3\pm 0.4\\ 3.5\pm 0.3\\ 7.1\pm 1.1\\ 219.2\pm 8.2\\ 368.1\pm 18.0\\ 128.2\pm 8.4\\ 63.9\pm 3.9\\ 6.9\pm 0.4 \end{array}$	$\begin{array}{c} 64.2\pm0.8\\ 103.2\pm5.4\\ 63.5\pm3.3\\ 43.9\pm6.4\\ 667.0\pm41.2\\ 84.5\pm1.8\\ \mathrm{tr}\\ 26.3\pm1.2\\ 28.2\pm4.4\\ 26.0\pm0.8\\ 1424.5\pm68.6\\ 2531.3\pm123.8\\ 962.1\pm57.5\\ 509.6\pm25.8\\ 64.2\pm0.8\\ \end{array}$	$58.8 \pm 3.1 \\ 86.0 \pm 4.2 \\ 56.8 \pm 8.4 \\ 44.3 \pm 5.9 \\ 561.2 \pm 26.8 \\ 80.8 \pm 9.9 \\ \mathrm{tr} \\ 23.7 \pm 1.1 \\ 21.6 \pm 1.7 \\ 21.9 \pm 3.0 \\ 1148.7 \pm 46.8 \\ 2103.7 \pm 103.3 \\ 829.1 \pm 50.6 \\ 439.3 \pm 22.5 \\ 58.8 \pm 3.1 \\ \end{array}$	$\begin{array}{c} 82.1\pm 3.6\\ 235.8\pm 12.1\\ 101.5\pm 4.0\\ 65.8\pm 0.9\\ 1510.7\pm 77.0\\ 136.3\pm 3.2\\ \mathrm{tr}\\ 40.5\pm 1.6\\ 36.5\pm 1.9\\ 21.3\pm 0.5\\ 2463.5\pm 145.6\\ 4694.0\pm 238.8\\ 2050.1\pm 92.2\\ 1051.1\pm 48.9\\ 82.1\pm 3.6\end{array}$	
$\alpha$ -galactosides (% of total) moisture (g/100 g of FW)	$\begin{array}{c} 42\\ 12.3\pm0.2\end{array}$	$\begin{array}{c} 38\\11.4\pm0.2\end{array}$	$\begin{array}{c} 40\\ 10.0\pm0.2 \end{array}$	$\begin{array}{c} 41\\ 9.0\pm0.2\end{array}$	$\begin{array}{c} 45\\ 11.6\pm0.3\end{array}$	

<sup>*a*</sup> Values are mean  $\pm$  SE of the mean. tr, trace.

 Table 2. Fagopyritol and Other Soluble Carbohydrate Composition of Buckwheat (Cv. Manor) Milling Fractions from Groats<sup>a</sup>

	buckwheat milling fractions from groats (mg/100 g of dry wt)						
soluble carbohydrate	whole groats (lot 1, 4/97)	Supreme flour (9/97)	Fancy flour (6/97)	Fancy flour (9/97)	bran (6/97)	bran (coarse ground, 9/97)	bran (fine ground, 9/97)
D- <i>chiro</i> -inositol	$26.5\pm0.6$	$39.3\pm0.7$	$16.6\pm0.5$	$15.4\pm1.2$	$94.5\pm3.1$	$112.0\pm2.5$	$161.7\pm4.0$
fagopyritol A1	$56.3\pm4.2$	$84.5\pm6.8$	$42.5\pm0.6$	$18.8 \pm 1.0$	$307.9 \pm 12.6$	$327.5 \pm 17.5$	$307.1 \pm 12.3$
fagopyritol A2	$36.3\pm3.0$	$46.9\pm2.5$	$24.3\pm1.1$	$14.2\pm2.3$	$138.0\pm8.2$	$152.6 \pm 16.4$	$188.8 \pm 19.2$
fagopyritol A3	$26.3\pm3.3$	$33.1\pm2.4$	$18.5\pm1.2$	$11.2\pm1.3$	$77.0\pm7.9$	$93.6\pm9.0$	$92.3\pm0.4$
fagopyritol B1	$392.5\pm27.4$	$518.4 \pm 39.0$	$258.2\pm4.4$	$115.7\pm5.4$	$1885.4\pm62.4$	$2004.0\pm85.5$	$1795.9 \pm 105.5$
fagopyritol B2	$53.3\pm4.7$	$57.9\pm0.6$	$31.8 \pm 0.9$	$16.5\pm1.2$	$177.3\pm8.4$	$196.8 \pm 18.8$	$216.0\pm26.6$
fagopyritol B3	tr	tr	tr	tr	tr	tr	tr
<i>myo</i> -inositol	$15.8\pm0.5$	$21.3\pm0.8$	$12.6\pm2.0$	$8.4\pm1.3$	$54.9 \pm 4.0$	$73.3\pm9.8$	$88.1 \pm 6.5$
galactinol	$20.6\pm1.5$	$21.0\pm1.1$	$6.8\pm0.9$	$5.2\pm0.8$	$52.4\pm6.2$	$70.4 \pm 4.3$	$67.8\pm3.9$
digalactosyl- <i>myo</i> -inositol	$13.2\pm1.1$	$14.0\pm0.3$	$6.0\pm0.2$	$4.5\pm0.6$	$33.7\pm0.6$	$36.4\pm2.6$	$43.6\pm7.2$
sucrose	$970.0\pm34.4$	$1027.5\pm82.9$	$474.2 \pm 11.5$	$223.0\pm7.0$	$3375.9\pm133.6$	$3876.3\pm95.7$	$3468.2 \pm 159.2$
total soluble carbohydrates	$1610.7\pm71.7$	$1863.9\pm127.4$	$891.5\pm19.6$	$432.9 \pm 17.0$	$6196.9\pm226.0$	$6943.0\pm226.8$	$6429.4\pm335.4$
total fagopyritols	$564.6 \pm 39.4$	$740.8 \pm 44.2$	$375.2\pm6.8$	$176.4\pm9.9$	$2585.6\pm86.5$	$2774.5 \pm 114.2$	$2600.1 \pm 158.1$
total D- <i>chiro</i> -inositol	$287.3 \pm 17.4$	$384.0\pm21.7$	$190.3\pm3.5$	$95.7\pm5.4$	$1315.5\pm41.2$	$1417.6\pm57.6$	$1371.2\pm76.0$
free D- <i>chiro</i> -inositol	$26.5\pm0.6$	$39.3\pm0.7$	$16.6\pm0.5$	$15.4 \pm 1.2$	$94.5\pm3.1$	$112.0\pm2.5$	$161.7\pm4.0$
$\alpha$ -galactosides (% of total)	37	42	44	43	43	41	42
moisture (g/100 g of FW)	$11.8\pm0.3$	$13.2\pm0.1$	$12.6\pm0.5$	$14.2\pm0.3$	$10.2\pm0.4$	$12.8\pm0.4$	$12.6\pm0.5$

<sup>*a*</sup> Values are mean  $\pm$  SE of the mean. tr, trace.

total soluble carbohydrates in tartary buckwheat was similar to that in common buckwheat cv. Manor, but only 21% of the total soluble carbohydrates were fagopyritols in tartary buckwheat (Table 3). Fagopyritol B1 was the primary fagopyritol in tartary buckwheat but was 56% of the total fagopyritols compared to 70% in common buckwheat.

An unknown compound was 31% of total soluble carbohydrates in groats of tartary buckwheat but was not present in groats of either cultivar of common buckwheat. The unknown compound appeared to be a dimer on the basis of its retention time relative to that of sucrose on gas chromatograms. Acid hydrolysis of the partially purified unknown compound yielded glucose and rhamnose (confirmed by GC coelution with authentic standards and by GC-MS). The unknown compound is tentatively identified as O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)-D-glucopyranose, the disaccharide unit of rutin, a flavonoid that is more concentrated in tartary buckwheat than in common buckwheat (Kitabayashi et al., 1995). Indeed, signals with retention times identical to that of authentic quercetin (the aglycon of rutin) were observed on gas chromatograms of tartary buckwheat extracts but not on those of common buckwheat extracts.

## DISCUSSION

Fagopyritols are concentrated in aleurone and embryo cells of the seed, but not in the pericarp (hull), seed coat, or starchy endosperm. Milling fractions originating from achenes, and therefore containing fragments of hull, have lower concentrations of fagopyritols than milling fractions from which the hull has been removed, due to dilution with tissues deficient in fagopyritols. Among milling fractions, bran has a high concentration of embryo and aleurone fragments. In the absence of hulls, bran fractions have the highest concentration of fagopyritols and total D-*chiro*-inositol among all milling fractions, reflecting the high amount of embryo and aleurone tissues in bran.

By contrast, embryo tissues in Fancy flour were diluted by the large proportion of starch (65-75%; Steadman et al., Cornell University, unpublished results). Because whole groats (4/97) include the entire

 Table 3. Fagopyritol and Other Soluble Carbohydrate Composition of Mature Groats from Two Cultivars (Manor and Mancan) of Common Buckwheat and from Tartary Buckwheat<sup>a</sup>

	mg/100 g of dry wt)					
soluble carbohydrate	Manor (lot 2)	Mancan (lot 1)	Mancan (lot 2)	tartary		
D- <i>chiro</i> -inositol	$20.7\pm1.1$	$41.7\pm3.5$	$36.7\pm3.1$	$23.5\pm1.0$		
fagopyritol A1	$43.8\pm2.7$	$47.6\pm3.3$	$35.2\pm4.6$	$67.3\pm6.4$		
fagopyritol A2	$36.8\pm4.9$	$10.0 \pm 1.0$	$11.3 \pm 1.4$	$36.5\pm25.5$		
fagopyritol A3	$37.8 \pm 3.3$	$23.3\pm0.1$	$24.3 \pm 1.1$	$43.0\pm9.3$		
fagopyritol B1	$300.4\pm25.7$	$242.7 \pm 16.7$	$186.5\pm33.2$	$210.2\pm8.1$		
fagopyritol B2	$45.9\pm5.5$	$9.6\pm0.7$	$12.1\pm3.1$	$20.5\pm6.6$		
fagopyritol B3	tr	tr	tr	0		
<i>myo</i> -inositol	$24.6\pm0.5$	$19.6 \pm 1.2$	$12.7\pm0.9$	$14.0\pm0.8$		
galactinol	$15.1 \pm 1.4$	$4.2\pm0.2$	$5.1 \pm 1.0$	$10.6\pm0.9$		
digalactosyl- <i>myo</i> -inositol	$13.5\pm3.0$	$2.6\pm0.1$	$3.7\pm0.2$	$12.3\pm3.0$		
sucrose	$744.7\pm86.9$	$381.8 \pm 11.8$	$395.3\pm78.3$	$792.2\pm76.1$		
rhamnosyl glucoside	0	0	0	$556.6 \pm 92.4$		
total soluble carbohydrates	$1283.4 \pm 128.5$	$783.0\pm29.7$	$722.9 \pm 125.7$	$1786.7\pm224.8$		
total fagopyritols	$464.7\pm39.1$	$333.2\pm21.4$	$269.4 \pm 43.3$	$377.6\pm55.5$		
total D- <i>chiro</i> -inositol	$229.8 \pm 17.0$	$199.2 \pm 13.9$	$161.5\pm23.1$	$192.0\pm22.4$		
free D- <i>chiro</i> -inositol	$20.7 \pm 1.1$	$41.7\pm3.5$	$36.7\pm3.1$	$23.5\pm1.0$		
$\alpha$ -galactosides (% of total)	38	43	39	22		
moisture (g/100 g of FW)	$10.1\pm0.2$	$11.2\pm0.2$	$9.0\pm0.1$	$9.7\pm0.3$		
dry wt (mg/seed)	$18.9\pm0.1$	$23.3\pm0.1$	$17.2\pm0.1$	$12.3\pm0.0$		

<sup>*a*</sup> Values are mean  $\pm$  SE of the mean. tr, trace.

embryo, aleurone, seed coat, and starchy endosperm, fagopyritol concentration was intermediate to that in Fancy flour and bran.

Bran (hulls removed, 2/97) derived by removing hull fragments from the milled achenes and bran (6/97 and 9/97) derived by dehulling achenes before the groats were milled theoretically should be equivalent in relative tissue components. Indeed, protein, lipid, and starch concentrations were similar (Steadman et al., Cornell University, unpublished results), but the concentrations of soluble carbohydrates were not the same. The temperature of the seed maturation environment is known to alter the composition of fagopyritols in buckwheat embryos. For example, both endosperm mass and fagopyritol B1 content are greatly enhanced in seeds matured at 18 °C compared to those matured at 25 °C (Horbowicz et al., 1998). Therefore, we compared seed from different lots, cultivars, and species. Because different seed lots were used to provide the milling fractions derived from achenes and those derived from groats, differences in seed maturation environments may have influenced the composition directly through fagopyritol accumulation and indirectly through the ratio of embryo to endosperm in the mature groat. Differences in carbohydrate concentration and composition between seed lots of common buckwheat may be the result of environmental effects. Differences between cultivars may be due to genetic and/or environmental factors. Genetic differences are more likely to be responsible for the observed differences in carbohydrates between species, that is, common buckwheat versus tartary buckwheat.

Buckwheat is unlike most plant seeds in its accumulation of  $\alpha$ -galactosyl D-*chiro*-inositols (fagopyritols) instead of the  $\alpha$ -galactosyl sucrose series (raffinose, stachyose, and verbascose) (Horbowicz and Obendorf, 1994). For the seed, these soluble carbohydrates may play a role in desiccation tolerance and storability (Horbowicz and Obendorf, 1994; Horbowicz et al., 1998).

Fagopyritols, galactosyl derivatives of D-*chiro*-inositol, are readily hydrolyzed by  $\alpha$ -galactosidase, releasing D-*chiro*-inositol (Horbowicz et al., 1998). Consumption of D-*chiro*-inositol may benefit persons with NIDDM through improved postprandial glycemic control. Feed-

ing D-chiro-inositol and D-pinitol (1D-3-O-methyl-chiroinositol) to humans increases plasma D-chiro-inositol and D-pinitol and lowers plasma glucose in subjects with NIDDM (Ostlund and Sherman, 1998). Feeding D-chiroinositol to hyperinsulinemic rhesus monkeys decreases plasma glucose (Ortmeyer et al., 1995). D-chiro-Inositol, administered orally, increases the action of insulin in patients with the polycystic ovary syndrome, thereby improving ovulatory function and decreasing serum androgen concentrations, blood pressure, and plasma triglyceride concentrations (Nestler et al., 1999). These reports provide evidence of uptake of D-chiro-inositol by the digestive system. We suggest that buckwheat, especially buckwheat bran (Farinetta), provides an edible source of fagopyritols, galactosyl derivatives of D-chiro-inositol, for future studies. The fate of fagopyritols in the human digestive system is unknown and requires further investigation.

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### LITERATURE CITED

- AOAC. *Official Methods of Analysis*, 16th ed., 3rd revision; AOAC International: Gaithersburg, MD, 1997.
- Asplin, I.; Galasko, G.; Larner, J. *chiro*-Inositol Deficiency and Insulin Resistance. A Comparison of the *chiro*-Inositol and the *myo*-Inositol-containing Insulin Mediators Isolated from Urine Hemodialysate and Muscle of Control and Type II Diabetic Subjects. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5924–5928.
- Berlin, W. K.; Wang, S. N.; Shen, T. Y. Glycosyl Inositol Derivatives. II. Synthesis of 2-Amino-2-deoxy-D-galactosyl- $\alpha$ -(1 $\rightarrow$ 3)-D-*chiro*-inositol. *Tetrahedron Lett.* **1990**, *31*, 1109–1112.
- Hansen, B. C.; Ortmeyer, H. K. Inositols—Potential Roles in Insulin Action and in Diabetes: Evidence from Insulinresistant Nonhuman Primates. In *Lessons from Animal Diabetes VI*; Shafrir, E., Ed.; Birkhaeuser: Boston, MA, 1996; pp 333–348.
- Horbowicz, M.; Obendorf, R. L. Seed Desiccation Tolerance and Storability: Dependence on Flatulence-producing Oligosac-

charides and Cyclitols–Review and Survey. *Seed Sci. Res.* **1994**, *4*, 385–405.

- Horbowicz, M.; Brenac, P.; Obendorf, R. L. Fagopyritol B1, O- $\alpha$ -D-Galactopyranosyl-(1 $\rightarrow$ 2)-D-*chiro*-inositol, a Galactosyl Cyclitol in Maturing Buckwheat Seeds Associated with Desiccation Tolerance. *Planta* **1998**, *205*, 1–11.
- Kennington, A. S.; Hill, C. R.; Craig, J.; Bogardus, C.; Raz, I.; Ortmeyer, H. K.; Hansen, B. C.; Romero, G.; Larner, J. Low Urinary *chiro*-Inositol Excretion in Non-insulin-dependent Diabetes Mellitus. *N. Engl. J. Med.* **1990**, *323*, 373–378.
- Kitabayashi, H.; Ujihara, A.; Hirose, T.; Minami, M. On the Genetic Differences for Rutin Content in Tartary Buckwheat, *Fagopyrum tataricum* Gaertn. *Breeding Sci.* 1995, 45, 189–194.
- Kornienko, A.; Marnera, G.; D'Alarcao, M. Synthesis of a Jojoba Bean Disaccharide. *Carbohydr. Res.* **1998**, *310*, 141– 144.
- Larner, J.; Huang, L. C.; Schwartz, C. F. W.; Oswald, A. S.; Shen, T. Y.; Kinter, M.; Tang, G.; Zeller, K. Rat Liver Insulin Mediator which Stimulates Pyruvate Dehydrogenase Phosphatase Contains Galactosamine and D-chiro-Inositol. Biochem. Biophys. Res. Commun. 1988, 151, 1416–1426.
- Lu, C.; Xu, J.; Žho, P.; Ma, H.; Tong, H.; Jin, Y.; Li, S. Clinical Application and Therapeutic Effect of Composite Tartary Buckwheat Flour on Hyperglycemia and Hyperlipidemia. In *Proceedings of the 5th International Symposium on Buckwheat*, Aug 20–26, 1992, Taiyuan, China; Lin, R., Zhou, M., Tao, Y., Li, J., Zhang, Z., Ed.; Agriculture Publishing House: Beijing, China, 1992; pp 458–464.
- Nestler, J. E.; Jakubowicz, D. J.; Reamer, P.; Gunn, R. D.; Allan, G. Ovulatory and Metabolic Effects of *D-chiro*-Inositol in the Polycystic Ovary Syndrome. *N. Engl. J. Med.* **1999**, *340*, 1314–1320.
- Ogawa, K.; Watanabe, T.; Ikeda, Y.; Kondo, S. A New Glycoside, 1D-2-O-α-D-Galactopyranosyl-chiro-inositol from Jojoba Beans. Carbohydr. Res. 1997, 302, 219–221.
- Ortmeyer, H. K.; Larner, J.; Hansen, B. C. Effects of D-chiro-Inositol Added to a Meal on Plasma Glucose and Insulin in Hyperinsulinemic Rhesus Monkeys. *Obesity Res.* 1995, 3 (Suppl. 4), 605S-608S.
- Ostlund, R. E., Jr.; Sherman, W. R. Pinitol and Derivatives Thereof for the Treatment of Metabolic Disorders. U.S. Patent 5,827,896, Oct 27, 1998; 13 pp.

- Ostlund, R. E., Jr.; McGill, J. B.; Herskowitz, I.; Kipnis, D. M.; Santiago, J. V.; Sherman, W. R. D-chiro-Inositol Metabolism in Diabetes Mellitus. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9988–9992.
- Pak, Y.; Hong, Y. G.; Kim, S.; Piccariello, T.; Farese, R. V.; Larner, J. In Vivo *chiro*-Inositol Metabolism in the Rat: A Defect in *chiro*-Inositol Synthesis from *myo*-Inositol and an Increased Incorporation of *chiro*-[<sup>3</sup>H]Inositol into Phospholipid in the Goto-Kakizaki (GK) Rat. *Molecules Cells* **1998**, *8*, 301–309.
- Quemener, B.; Brillouet, J. M. Ciceritol, a Pinitol Digalactoside from Seeds of Chickpea, Lentil and White Lupin. *Phy*tochemistry **1983**, 22, 1745–1751.
- Schweizer, T. F.; Horman, I. Purification and Structure Determination of Three  $\alpha$ -D-galactopyranosyl Cyclitols from Soya Bean. *Carbohydr. Res.* **1981**, *95*, 61–71.
- Shashkin, P. N.; Shashkina, E. F.; Fermqvist-Forbes, E.; Zhou, Y. P.; Grill, V.; Katz, A. Insulin Mediators in Man: Effects of Glucose Ingestion and Insulin Resistance. *Diabetologia* **1997**, 40, 557–563.
- Shiomi, N.; Takeda, T.; Kiriyama, S. A New Digalactosyl Cyclitol from Seed Balls of Sugar Beet. *Agric. Biol. Chem.* 1988, *52*, 1587–1588.
- Szczeciński, P.; Gryff-Keller, A.; Horbowicz, M.; Obendorf, R. L. NMR Investigation of the Structure of Fagopyritol B1 from Buckwheat Seeds. *Bull. Pol. Acad. Sci., Chem.* **1998**, 46, 9–13.
- Wang, J.; Liu, Z.; Fu, X.; Run, M. A Clinical Observation on the Hypoglycemic Effect of Xinjiang Buckwheat. In *Proceedings of the 5th International Symposium on Buckwheat*, Aug 20–26, 1992, Taiyuan, China; Lin, R., Zhou, M., Tao, Y., Li, J., Zhang, Z., Eds.; Agriculture Publishing House: Beijing, China, 1992; pp 465–467.

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