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Lentil (*Lens culinaris* L.): A prebiotic-rich whole food legume

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ABSTRACT

Prebiotic carbohydrates are important components of healthy diets, supporting healthful hindgut microflora. Lentils grown in North Dakota, USA were evaluated for their prebiotic carbohydrates. Raffinose-family oligosaccharides (RFO), sugar alcohols, fructooligosaccharides (FOS), and resistant starch (RS) carbohydrates were analyzed in 10 commercial lentil varieties grown in Ward and McLean Counties in 2010 and 2011. Mean concentrations of RFO, sugar alcohols, FOS and RS were 4071 mg, 1423 mg, 62 mg, and 7.5 g 100 g^{-1} dry matter, respectively. Significant variations were observed in lentil prebiotic carbohydrate concentrations: RFO concentrations varied with variety, RS varied with location, and sorbitol and mannitol each varied with both variety and location. These results show that lentils contain nutritionally significant amounts of prebiotic carbohydrates and, that it may be possible to enhance those amounts through breeding and locational sourcing.

1. Introduction

Obesity and related non-communicable diseases are of global concern, affecting more than one in every ten adults (World Health Organization, 2012). The prevalence of obesity in the United States is estimated to be over 35% among adults (Flegal, Carroll, Kit, & Ogden, 2012). Chronic, non-communicable diseases associated with obesity, including diabetes, cardiovascular diseases, and some types of cancer, result in an estimated 36 million deaths globally each year, claiming more lives than all other causes of death combined (United Nations, 2012). Due to the dietary nature of these metabolic disorders, solutions will necessarily have a focus on diet.

Prebiotics may contribute to dietary strategies to reduce obesity (Cani et al., 2009; Parnell & Reimer, 2009). Roberfroid offered a revised definition of a prebiotic: "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (Roberfroid, 2007). Such changes among microbial species colonies in the human gut can produce a wide range of positive effects, including increased satiety, regulation of the intestinal motility, production of short-chain fatty acids, prevention of diarrhea and constipation, and reduction of pathogen colonization (Caselato, Freitas, & Sgarbieri, 2011; Manning & Gibson, 2004; Scheppach, Luehrs, & Menzel, 2001). Moreover, consumption of prebiotics may stimulate the immune system (Lee & Mazmanian, 2010), promote mineral absorption, decrease risk of colon cancer (Burns & Rowland, 2000; Conlon et al., 2012; Rowland, 2009), and decrease risk factors associated with obesity and metabolic syndrome (Brugman et al., 2004; Caselato et al., 2011; Rabot et al., 2010). Prebiotics have been shown to reduce excess circulating glucose and cholesterol levels (Kaur & Gupta, 2002) and improve insulin sensitivity (Johnston, Thomas, Bell, Frost, & Robertson, 2010).

Naturally occurring prebiotic carbohydrates are in the larger category of dietary fiber, and, as defined by the Institute of Medicine, dietary fiber is nondigestible carbohydrate and lignin intrinsic to plants (Report of the Panel on Macronutrients Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). The European Food Standard Agency set the Dietary Reference Value for dietary fiber at 25 g per day for adults 18 years of age or older to sustain normal bowel function but acknowledged that higher intakes could provide additional benefits (European Food Safety Authority, 2010). However, a National Health and Nutrition Examination Survey (NHANES) found Americans 20 years of age and older consume only 61% of the indicated level (U.S. Department of Agriculture, 2010). While official recommendations have not been made regarding prebiotic consumption, several investigators have offered suggestions: 10 g per day of fructooligosaccharide (FOS) (Bouhnik et al., 1999) and 7 g per day of galactooligosaccharide (GOS) (Silk, Davis, Vulevic, Tzortzis, & Gibson, 2009). Resistant starch (RS) may elicit effects at low intake levels, but investigators have shown that consumption of up to 45 g per day is well-tolerated (van den Heuvel et al., 2004). Average consumption of prebiotics is estimated to be several grams per day (Moshfegh, Friday, Goldman, & Ahuja, 1999; van Loo, Coussement, de Leenheer, Hoebregs, & Smits, 1995), which is indicative of the low levels of prebiotic compounds in most commonly eaten foods in the Western diet.

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An overlooked yet potential source of prebiotic carbohydrates is lentil (*Lens culinaris* L.), a widely grown grain legume and dietary staple in many Middle Eastern, European, South American, African and Asian countries. Lentils are known to contain GOS, which include raffinose family oligosaccharides (RFO) (Bhatty, 1988). Prebiotic effects of GOS, primarily via bifidogenesis, include increased calcium absorption and pathogen reduction (Brouns & Vermeer, 2000; Scholtens et al., 2006). Resistant starch, which is well-documented in lentil (Chung et al., 2008; de Almeida Costa, da Silva Queiroz-Monici, Pissini Machado Reis, & de Oliveira, 2006; Wang, Hatcher, Toews, & Gawalko, 2009), improved insulin sensitivity in men with metabolic syndrome on a high RS diet (Johnston et al., 2010). Fructooligosaccharides, such as kestose and nystose, are well-known for their prebiotic action (Gibson & Roberfroid, 1995; Scholtens et al., 2006; van Loo et al., 1999). Sugar alcohols have been shown to displace pathogens from rumen and gastrointestinal tract and increase viability of strains of *Bifidobacteria* and *Lactobacilli* (de Vaux, Morrison, & Hutkins, 2002; Yeo & Liong, 2010). Sorbitol, mannitol, kestose, and nystose were not detected in lentils grown in Australia (Biesiekierski et al., 2011), although sorbitol was reported in varying concentrations among germinated seeds of lentil varieties (Asghar, Stushnoff, & Johnson, 2000). Some prebiotic carbohydrates show significant variation among lentil varieties, suggesting potential for increasing their amounts through conventional plant breeding (Chung et al., 2008; de Almeida Costa et al., 2006; Tahir, Vandenberg, & Chibbar, 2011; Wang et al., 2009).

Though some research has been devoted to prebiotic compounds in lentil, focus has not been toward these compounds as prebiotics, and the scope of the previously analyzed carbohydrates has been narrow. To our knowledge, no study has extensively examined the prebiotic profile in lentil varieties in a replicated field study. The objectives of the present study were to (1) characterize the prebiotic carbohydrate profile [fructooligosaccharide (kestose and nystose), raffinose family sugars (raffinose, stachyose, and verbascose), sugar alcohols (sorbitol and mannitol), total starch, and resistant starch] of US grown lentil varieties; and (2) determine the genetic and environment variation in lentil prebiotic carbohydrates.

2. Materials and methods

2.1. Materials

Standards, reagents, and high-purity solvents used for high-performance liquid chromatographic (HPLC) analyses and enzymatic assays were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and VWR International (Radnor, PA, USA) and were used without further purification. Regular maize starch (Megazyme International Ireland Ltd., Bray, Ireland) was used as an external reference sample. Water, distilled and deionized (ddH_20) to a resistance of ≥18.2 MΩ (Milli-Q Water System, Millipore, Milford, MA), was used for sample extractions and preparation.

2.2. Lentil samples

Seeds from ten commercial lentil varieties (Table 1) were obtained from a regional variety trial conducted in 2010 and 2011 by the Pulse Breeding Program at North Dakota State University, North Dakota, USA. Subsamples of seeds for HPLC analysis of soluble carbohydrates and determination of RS were randomly taken from entire harvested plot of each of three replicated randomized field plots at two locations, Ward (48.2325° N, 101.2958° W) and McLean (47.5774° N, 101.2360° W) Counties, for both years. Subsamples (10–20 g of seed; 7.3% moisture) were stored at −40 °C until analysis. Samples were cleaned of debris and ground to pass through a sieve size of 0.25 mm using a top-loading UD grinder (Unholtz Dickie Corporation, USA).

Table 1
Market class, major consuming countries, and 1000 seed weight of 10 lentil varieties grown in North Dakota, USA.

Market class	Major consuming countries[a]	Variety	1000 seed weight (g)[b]
Extra small red	Bangladesh, Pakistan, Egypt	CDC Rosetown	26 *h*
Small red	Southern Asia, the Middle East, northern Africa	CDC Red Rider	40 *e*
		CDC Redberry	38 *e*
		CDC Rouleau	34 *f*
Small green	Morocco, Greece, Italy, Egypt, Mexico	CDC Viceroy	29 *g*
Medium green	North-western Europe, Spain, Algeria, United States	CDC Richlea	43 *d*
Large green	North-western and southern Europe, Algeria, South America, and Central America	Pennell	59 *b*
		Riveland	62 *a*
		CDC Greenland	56 *c*
Dark green speckled	France	CDC Lemay	30 *g*

[a] Data obtained from Thavarajah, Ruszkowski and Vandenberg (2008).
[b] Means followed by the same letter within a column are not significantly different at $p<0.05$. Standard error for 1000 seed weight is 0.2 g.

2.3. Analysis of water soluble prebiotic carbohydrates

Water soluble prebiotic carbohydrates for each replicated lentil sample were extracted using a method described by Muir et al. (2009). Each ground sample (500 mg) was weighed into a 15 mL polystyrene conical tube. Samples were dissolved in 10 mL of ddH_2O and incubated in an 80 °C water bath for 1 h, then centrifuged at 3000× *g* for 10 min using a Beckman GPR centrifuge (Fullerton, CA, USA). After centrifugation, a 1 mL aliquot of the supernatant was diluted with 10 mL of ddH_2O and passed through a 13 mm×0.45 μm nylon syringe filter (Chromatographic Specialties, Brockville, ON). Extraction and chemical analysis of oligosaccharides and sugar alcohols was performed on a Dionex system (ICS-5000 Dionex, Sunnyvale, CA, USA) using a method previously described by Feinberg, San-Redon, and Assie' (2009). Oligosaccharides were separated using a CarboPac PA1 column (250×4 mm; Dionex, Sunnyvale, CA, USA) in series with a CarboPac PA1 guard column (50×4 mm). The mobile phase flow rate was maintained at 1 mL/min. Solvents used for elution were 100 mM sodium hydroxide/600 mM sodium acetate (solvent A), 200 mM sodium hydroxide (solvent B), and 18 MΩ deionized water (solvent C). Solvents B and C at 50% each were used for an initial 2 min, followed by a linear gradient change from 2% A, 49% B, and 49% C at 2 min to 16% A, 42% B, and 42% C at 20 min. The final interval resumed initial conditions of 50% B and 50% C. Detection of oligosaccharides was carried out using a pulsed amperometric detector (PAD) with a working gold electrode with a silver–silver chloride electrode at 2.0 μA. Carbohydrate concentrations reported in the current study were identified based on the pure standards obtained from Sigma Aldrich Chemical Company. The concentrations of those analyzed carbohydrates were detected within a linear range of 3–100 μg/g. The minimal detectible limit was 0.2 μg/g. An external lab reference, CDC Redberry, was also used daily to ensure accuracy and reproducibility of detection. Oligosaccharide peak areas for the reference sample were routinely analyzed with an error of less than 5%. Standard solutions of prebiotic carbohydrates were prepared for peak identification and run daily to ensure detection sensitivity. Linear calibration models for oligosaccharide standards had an error of less than 4%. Concentrations of oligosaccharides in the filtrate (C) were calculated from the calibration model used to calculate concentrations in sample dry matter in the expression $X=(C\times V)/m$, where X is the concentration of oligosaccharide in the sample (corrected for moisture), V is the final diluted volume, and m is the mass of the dry sample aliquot.

2.4. Resistant starch analysis

Resistant starch analysis was performed by a method approved by AOAC International, previously described (McCleary & Monaghan,

2002; Megazyme, 2012). This involved incubating 50 mg ground lentil seed with 2 mL of a solution containing amyloglucosidase (3 U/mL) and α-amylase (10 mg/mL) in 100 mM sodium maleate (pH 6.0) at 37 °C for 16 h with constant circular shaking. Samples were then washed with 2 mL ethanol (≥95% pure), and again centrifuged at 3000× *g* for 13 min at room temperature (RT). Pellets were re-suspended with 4 mL of 50% ethanol (v:v), centrifuged, and decanted two additional times. Washings from the three centrifugations were pooled and brought to a volume of 50 mL with distilled water. Pellets containing the resistant starch fraction were dissolved with 1 mL of 2 M KOH with stirring at 4 °C for 20 min. After dissolution of the RS, 4 mL of 1.2 M sodium acetate buffer (pH 3.8) and 0.5 mL of amyloglucosidase (300 U/mL) were introduced into the tubes, which were incubated at 50 °C for 30 min with intermittent stirring. Samples were then centrifuged (3× *g* for 13 min at RT) and 100 μL aliquots (in duplicate) of both the supernatant containing the RS fractions and the diluted washings containing the soluble starch fractions were transferred to 15 mL polystyrene tubes. A reagent blank was prepared using 100 μL dilute sodium acetate buffer (pH 4.5). Glucose standards (1 mg/mL) were prepared and 100 μL aliquots (in triplicate) were transferred to tubes. A 3 mL aliquot of a reagent containing glucose oxidase (>12,000 U/L), peroxidase (>650 U/L), and 4-aminoantipyrine (0.4 mM) at a pH of 7.4 was transferred to each tube. Tubes were incubated in a water bath at 50 °C for 20 min. Absorption at 510 nm was measured using a Shimadzu UV 1800 Spectrophotometer (Shimadzu, Japan).

Starch fractions were calculated using

$$\begin{aligned} \mathrm{NRS} &= \frac{x\left(\Delta A_{\mathrm{sample}}\right)}{\left(\Delta A_{\mathrm{glucose}}\right)\left(W_{\mathrm{sample}}\right)} \\ \mathrm{RS} &= \frac{y\left(\Delta A_{\mathrm{sample}}\right)}{\left(\Delta A_{\mathrm{glucose}}\right)\left(W_{\mathrm{sample}}\right)} \\ \mathrm{TS} &= \mathrm{RS} + \mathrm{NRS} \end{aligned} \quad (1)$$

where ΔA_{sample} and $\Delta A_{glucose}$ are the change in absorbance of sample and glucose, respectively as measured against reagent blank, W_{sample} is the weight of sample corrected for moisture, *x* is a factor to account for dilutions in determination of NRS, *y* is a factor to account for dilutions in determination of resistant starch, and total starch (TS) is the sum of RS and non-resistant starch (NRS). Analysis of resistant starch by this method routinely achieves a standard error of ±5% for samples that contain >2% resistant starch.

2.5. Statistical analysis

The experiment was a randomized complete block design with three replicates of ten commercial lentil varieties at two locations over two years ($n = 120$). Replicates, locations, and varieties were considered as random factors. Years, locations, varieties, and replicates were included as class variables. Data were analyzed in a combined model and separately by year and location. Analysis of variance was performed using the General Linear Model procedure (PROC GLM) of SAS version 9.2 (SAS Institute, 2009). Means were separated by Fisher's protected least significant difference (LSD) at $p<0.05$.

3. Results

3.1. Thousand seed weight

Table 1 provides 1000 seed weights of 10 lentil varieties and their respective market classes. Thousand-weights of varieties within the large green market class varied from 56 to 62 g per 1000-seed. Thousand seed weights for varieties of the small red market class ranged from 34 to 40 g per 1000-seed. The extra small red market class, CDC Rosetown, had a significantly lower 1000 seed weight (26 g per 1000-seed) compared to all other varieties. The medium green lentil, CDC Richlea, and the dark green speckled lentil, CDC Lemay, had 1000 seed weights of 43 and 30 g per 1000-seed, respectively. Combined statistical analysis reveals significant variance of 1000 seed weight by year, location, variety, replication, and the year × location interaction (Table 2). Significant replication effect was observed as a result of gradient of soil moisture or fertility or other unknown factors.

3.2. Concentrations of water soluble prebiotic carbohydrates

Table 3 shows mean concentration values of prebiotic carbohydrates and TS. Sorbitol concentrations ranged from 1.0 to 1.3% (dry weight basis) in lentils. The highest sorbitol concentration was observed in the variety Riveland (1349 mg 100 g^{-1}) and the lowest in CDC Red Rider (1036 mg 100 g^{-1}), CDC Lemay (1039 mg 100 g^{-1}), and CDC Greenland (1109 mg 100 g^{-1}). Combined statistical analysis reveals significant variance in sorbitol concentrations by year, location, and variety (Table 2). Mannitol accounted for less than 0.3% of dry lentil weight. The highest concentrations of mannitol were observed in CDC Richlea (294 mg 100 g^{-1}) and Riveland (248 mg 100 g^{-1}) compared to all other tested varieties (Table 3). The lowest concentrations of mannitol were observed in CDC Rosetown (158 mg 100 g^{-1}), CDC Red Rider (160 mg 100 g^{-1}), CDC Lemay (163 mg 100 g^{-1}), and CDC Redberry (176 mg 100 g^{-1}). Mannitol concentrations showed significant variance by year, location, variety, and the year × location interaction (Table 2).

To minimize variation due to weather, agricultural practices, and soil, data were also statistically analyzed by location and year (Table 4). Mean values of carbohydrate concentrations were taken from all samples within a location and year. Mean concentrations of sorbitol and mannitol were higher in lentils grown in McLean County vs. Ward County for both years. Mean sorbitol and mannitol concentrations were significantly higher in 2010 (1267 and 217 mg 100 g^{-1}, respectively) than in 2011 (1172 and 188 mg 100 g^{-1}, respectively).

Table 2
Combined analysis of variance for seed weight (TSW), sorbitol (Sorb), mannitol (Mann), raffinose (Raff), stachyose (Stach), verbascose (Verb), nystose (Nys), resistant starch (RS), and total starch (TS) for 10 lentil varieties grown in North Dakota, USA in 2010 and 2011.

Source	Mean square[a]								
	Df[b]	TSW	Sorb	Mann	Raff + Stach	Verb	Nys	RS	TS
Year	1	513**	23,919**	4023**	5349	40,429	237	2	269**
Location	1	941**	92,796**	7404**	2656	97,652**	40	52*	47**
Variety	8	2027**	14,284**	3566**	8834	73,239**	142	12	5
Replication (year, location)	9	14*	1534	88	649	3056	7	2	16
Year × location	1	1021**	1441	1796**	60	75,883**	136	192**	24
Year × variety	9	46	2573	342	7446**	17,598**	150	5	15
Location × variety	9	27	1133	721	2112	4001*	101	11	2
Year × location × variety	9	22**	5429**	306	1215	1549	139	5	11
Error	72	7	1378	94	1122	2100	8	3	4

[a] Mean square was significantly different at $p<0.05$ (**) and $p<0.1$ (*).
[b] Degrees of freedom based on three replicates.

Table 3
Mean concentration of prebiotic carbohydrates of 10 lentil varieties grown in North Dakota, USA, in 2010 and 2011.

Variety	mg 100 g^{-1}[a]				
	Sorb	Mann	Raff + Stach[b,c]	Verb	Nys[b]
CDC Greenland	1109 *c*	211 *c*	2426	1770 *b*	57
CDC Lemay	1039 *c*	163 *d*	2497	1495 *d*	57
CDC Red Rider	1036 *c*	160 *d*	2419	1586 *cd*	52
CDC Redberry	1226 *b*	176 *d*	2349	1481 *d*	61
CDC Richlea	1295 *ab*	294 *a*	2319	1731 *bc*	62
CDC Rosetown	1325 *ab*	158 *d*	2586	922 *e*	62
CDC Rouleau	1304 *ab*	199 *c*	2793	1082 *e*	63
CDC Viceroy	1285 *ab*	215 *c*	2530	1800 *b*	79
Pennell	1231 *b*	204 *c*	2684	1968 *a*	57
Riveland	1349 *a*	249 *b*	2492	1784 *b*	68
Mean	1220	203	2509	1562	62
SE	11.6	2.2	17	18	0.6

SE, standard error of combined data ($n = 120$). Sorb, sorbitol; Mann, mannitol; Raff, raffinose; Stach, stachyose; Verb, verbascose; Nys, nystose.
[a] Means within a column followed by different letters are significantly different at $p < 0.05$.
[b] Mean concentration of varieties are not significantly different.
[c] Raffinose and stachyose are reported as total raffinose and stachyose concentration due to similar elution times for the separation method.

Verbascose concentrations exhibited substantial variation between varieties, doubling from lowest- to highest-concentration varieties (Table 3). Verbascose levels were highest in Pennell (1968 mg 100 g^{-1}) and lowest in CDC Rosetown (922 mg 100 g^{-1}) and CDC Rouleau (1082 mg 100 g^{-1}). Variance of verbascose concentration was observed by location, variety, year × location, year × variety, and variety × location (Table 2). Raffinose and stachyose, reported as a mean, combined total, only showed variance for the interaction between year and variety. Raffinose and stachyose concentrations ranged from 2319 to 2793 mg 100 g^{-1} (Table 3). Analysis of raffinose, stachyose, and nystose did not reveal variation by variety (Table 2).

Within years and locations (Table 4), concentration values of RFO tended to be higher in McLean County than in Ward County. Mean verbascose concentrations were 1710 and 1656 mg 100 g^{-1} in lentils from McLean County and 1255 and 1627 mg 100 g^{-1} from Ward County in 2010 and 2011, respectively. Raffinose and stachyose concentrations were slightly but not significantly higher in lentils from McLean vs. Ward County and in 2010 vs. 2011. Mean verbascose concentrations were significantly higher in 2011 than in 2010.

Nystose, the only observed member of the fructooligosaccharide family, showed no variation that reached statistical significance under the combined model. Nystose concentrations ranged from 52 to 79 mg 100 g^{-1} and variance was only observed for location from 2011 data, when the mean concentration from McLean County (67 mg 100 g^{-1}) was higher than that from Ward County (61 mg 100 g^{-1}). Nystose was slightly higher in lentils from 2011 than those from 2010, but values were not statistically significant. Kestose was not detected.

Mean concentration values of prebiotics for all 10 lentil varieties from both locations and years are derived from Table 3 data. Total sugar alcohol concentrations, as expressed by the sum of sorbitol and mannitol, accounted for approximately 1.4% of dry lentil flour weight. Total sugar alcohol concentrations varied from 1196 mg 100 g^{-1} in the CDC variety Red Rider to 1598 mg 100 g^{-1} in the Riveland variety. Total RFO accounted for 4%, on average, of dry lentil flour weight. Concentrations of total RFO ranged from 3508 mg 100 g^{-1} in CDC Rosetown to 4652 mg 100 g^{-1} in Pennell. Total FOS comprised approximately 0.06% of dry lentil flour weight, ranging from 52 mg 100 g^{-1} in CDC Red Rider to 79 mg 100 g^{-1} in CDC Viceroy.

3.3. Concentrations of resistant starch and total starch

Resistant and total starch concentrations of the 10 lentil varieties are shown in Fig. 1. Mean concentrations of RS and TS for all samples were 7.5 and 47 g 100 g^{-1}, respectively. Resistant starch averages ranged from 6.0 g 100 g^{-1} in CDC Greenland to 8.9 g 100 g^{-1} in Pennell. Total starch ranged from 45 to 48 g 100 g^{-1}. Combined statistical analysis (Table 2) showed variance for resistant starch by location and the year × location interaction and for total starch by year and by location.

Starch data were also analyzed by year and location (Table 4). Resistant starch concentrations were higher in McLean County (9.3 g 100 g^{-1}) compared to Ward County (5.5 g 100 g^{-1}) in 2010 but higher in Ward County (8.3 g 100 g^{-1}) compared to McLean County (7.1 g 100 g^{-1}) in 2011. Total starch was higher in Ward County (46.5 g 100 g^{-1}) than in McLean County (44.4 g 100 g^{-1}) in 2011 but mean values were not significantly different in 2010. Overall mean TS concentrations were significantly higher in 2010 (48 g 100 g^{-1}) than in 2011 (45 g 100 g^{-1}).

4. Discussion

An understanding of prebiotic concentrations in lentils varieties could provide insight to allow for: A) selection of more nutritious lentil market classes; B) an opportunity to further improve overall lentil nutritional quality through breeding and food processing; and C) an understanding of environmental and genetic factors affecting prebiotic carbohydrates, allowing selection of optimal lentil growing locations for mass production. Variation of RFO (Tahir, Lindeboom, Baga, Vandenberg, & Chibbar, 2011; Tahir, Vandenberg, et al., 2011; Wang et al., 2009) and RS (Chung et al., 2008; de Almeida Costa et al., 2006; Wang et al., 2009) concentrations in several commercial lentil varieties have been reported, but these studies have not been designed to assess variation among varieties or environmental influences. Although sorbitol concentrations have been quantified in the shoots and basal leaves for several older lentil varieties not in production, mannitol concentrations were not examined (Asghar et al., 2000). To our knowledge, this is the first study to quantify RFO, RS, FOS, and sugar alcohols in lentils in a replicated field study.

Table 4
Mean concentrations of prebiotic carbohydrates and total starch by year and location.

Year	Location	mg 100 g^{-1}[a]					g 100 g^{-1}	
		Sorb	Mann	Raff + Stach	Verb	Nys	RS	TS
2010	McLean	1373 *x*	246 *x*	2566 *x*	1710 *x*	61 *x*	9.3 *x*	48. *x*
	Ward	1161 *y*	188 *y*	2524 *x*	1255 *y*	57 *x*	5.5 *y*	49 *x*
	Mean	1267	217	2545	1482	59	7.4	48
	SE	19	3.6	27	26	0.9	0.2	0.3
2011	McLean	1255 *x*	198 *x*	2503 *x*	1656 *x*	67 *x*	7.1 *y*	44 *y*
	Ward	1089 *y*	178 *y*	2444 *x*	1627 *y*	61 *y*	8.3 *x*	47 *x*
	Mean	1172	188	2474	1641	64	7.7	45
	SE	13.4	2.7	18	23.7	0.9	0.2	0.2

Sorb, sorbitol; Mann, mannitol; Raff, raffinose; Stach, stachyose; Verb, verbascose; Nys, nystose; RS, resistant starch; TS, total starch. SE, standard error ($n = 60$).
[a] Means within a column followed by different letters are significantly different at $p < 0.05$.

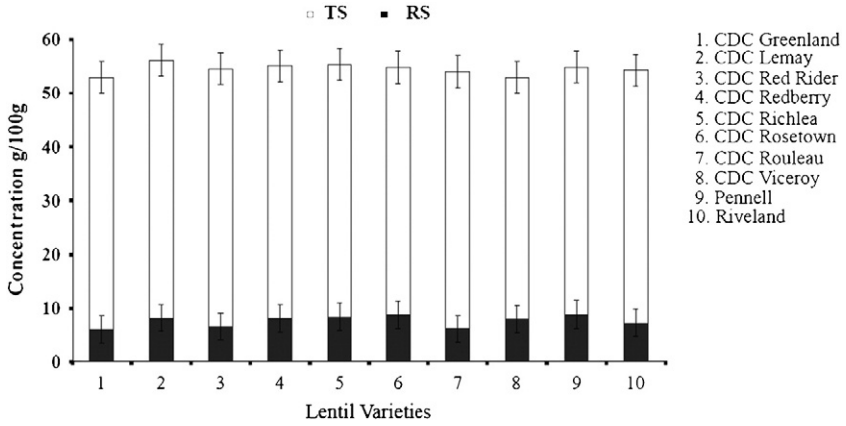

Fig. 1. Mean total starch and resistant starch concentrations of 10 lentil genotypes grown in North Dakota, USA in 2010 and 2011.

Mean concentrations of various prebiotic compounds have been reported in lentil. Raffinose-family oligosaccharides were first reported in the late 1970s-early 1980s [raffinose, 0.39–1.0% (dry weight basis); stachyose, 1.47–3.1%; verbascose, 0.47–3.1%] (Bhatty, 1988). More recent reports include similar ranges [raffinose, 0.47–2.0%; stachyose, 1.7–2.9%; verbascose, 0.7–1.9%] (Tahir, Vandenberg, et al., 2011; Wang et al., 2009) and compare to values from this study (raffinose/stachyose combined, 2.5%; verbascose, 1.6%). Mean total RFO from our study was 4.1%, which falls in the lower half of the range from previous reports (2.5–7.2%) (Bhatty, 1988; Wang et al., 2009). Other studies analyzing RFO concentrations of CDC Richlea have reported values either similar to (Wang et al., 2009) or higher than (0.5 to 1.5% percent of seed weight, dry; Tahir, Vandenberg, et al., 2011) our findings; such differences within the same variety may be due to environmental effects or differences in analytical procedures.

Resistant starch concentrations in raw and cooked lentils have been reported to range from 1.6 to 5.2% of dry lentil seed weight (Chung et al., 2008; Wang et al., 2009) and 1.6 to 9.1 g 100 g^{-1} of cooked lentils (Yanetz et al., 2008). These values are substantially lower than the present findings for dry lentils (Fig. 1). Current methods for quantification of resistant starch include in vitro assays performed with amyloglucosidase and alpha-amylase concentrations at the pH of the duodenum. Due to variability within the human digestive system, resistant starch is difficult to approximate. Concentrations of RS are also affected by cooking, processing, and cooling (Wang et al., 2009). Lentil is cooked before being consumed; making measurement of resistant starch in lentil flour nutritionally irrelevant, but analysis may be useful in comparison between lentil varieties for future breeding and selection.

Prebiotic concentrations in lentils appear to be related to genetic and environmental factors. Location significantly influenced concentrations of various prebiotics carbohydrates (Tables 2 and 4). In May of 2011, both Ward and McLean Counties were eligible for public assistance due to flood damage (Federal Emergency Management Agency, 2011). Soil data from Mandan, North Dakota, which lies in the same river basin where the field studies were located, indicates that percent soil moisture increased from 32% saturation (average of top 20 in. of soil) in 2010 to over 36% saturation in 2011 (National Resources Conservation Service, 2011). This was coincident with significant reductions in sorbitol, mannitol, and total starch concentrations in lentil grown in 2011 vs. 2010 (Table 4). Sorbitol and mannitol are humectants which can retain moisture, similar to corn starch that has a water binding capacity of 85–92% (Sandhu & Singh, 2006). Together, this information suggests that the lentil plants may decrease production of sugar alcohols and starch under stressful, high moisture conditions to avoid water saturation and decomposition of mature seeds, thus protecting seed viability for the following year.

Locational variance suggests that soil characteristics, moisture, and weather have a greater influence on resistant starch content than genetics. Conversely, the variety effect was significant with respect to concentrations of sorbitol, mannitol, and verbascose. While other studies have indicated significant variety effect on raffinose and stachyose concentrations (Tahir, Vandenberg, et al., 2011; Wang et al., 2009), our study did not reveal significant variation with variety, likely due to their concentrations being expressed as a combined total. Optimization of prebiotics in lentil varieties would necessarily have to consider both hereditary and environmental influences on prebiotic compounds.

Sugar alcohols, although influenced by the environment, also appear to be genetically-linked seed characteristics along with other prebiotics, including the RFOs (Table 2). Seed size, as measured by 1000-seed weight, was positively correlated to total water-soluble prebiotic carbohydrate concentration and inversely correlated to resistant starch (data not shown). Although seed size was positively correlated to the amount of soluble prebiotic carbohydrates, smaller seed sizes within market classes had higher concentrations of total soluble prebiotics than larger varieties. Seed size, therefore, is not a useful indicator of total prebiotic carbohydrate content. Total soluble prebiotic carbohydrates were 5753 mg 100 g^{-1} in green lentil market classes and 5260 mg 100 g^{-1} in red lentil market classes (data derived from Table 3). Resistant starch was slightly higher in green lentils (7.8 g 100 g^{-1}) than in red lentils (7.4 g 100 g^{-1}) (Fig. 1). Relative concentrations of prebiotic carbohydrates may be more closely linked to green- or red-cotyledon traits than seed size. All commercial lentil market classes were relatively high and uniform in total prebiotic carbohydrate concentrations. Total prebiotic concentrations in lentils ranged from 11.5 g 100 g^{-1} in CDC Rouleau to 15.0 g 100 g^{-1} in Pennell (data not shown). Concentrations of total prebiotic carbohydrates of these two varieties are consistent with their respective market classes, small red and large green, respectively (Table 5).

Our results indicate that lentil may be a good source of prebiotic carbohydrates. Total prebiotic carbohydrate concentrations suggest that a 100 g serving of lentils may provide over 13 g of prebiotics. In wheat (*Triticum* spp.) varieties, fructans range from 0.5 to 1.5% (Huynh et al., 2008) and RS from 1.5 to 2.5% (Bonafaccia et al., 2000). Based on this information, wheat varieties may contain from 2 to 4% prebiotic content as a raw grain. Average consumption of prebiotics is estimated to be several grams per day (Moshfegh et al., 1999; van Loo et al., 1995), which is indicative of the low levels of prebiotic compounds in most commonly eaten foods in the Western diet.

Future studies of the prebiotic carbohydrates in lentils are necessary to understand the physiological and environmental control of prebiotic carbohydrate expression. Of interest would be studies focusing on resistant starch concentrations in relation to soil and moisture characteristics. Moreover, processing, germination, and cooking are essential when evaluating lentil as a dietary source of prebiotics. RFO concentrations change with cooking (de Almeida Costa et al., 2006; Wang et al., 2009), with raffinose and stachyose decreasing

Table 5
Concentrations of total prebiotic carbohydrates, galactooligosaccharides (GOS), and resistant starch (RS) in a 100 g serving of lentils by market class with dietician recommended intake values.

Market class	Total prebiotic carbohydrate from 100 g serving (g)	Daily GOS intake from 100 g serving (g)	Daily RS intake from 100 g serving (g)
Extra small red	13.9	3.5	8.8
Small red	12.3	3.9	6.9
Small green	13.9	4.3	8.4
Medium green	14.1	4.1	8.0
Large green	13.3	4.4	7.4
Dark green speckled	13.5	4.0	8.2
Recommended prebiotic intake (g per day)	10–20 g per day[a]	2–7 g per day[b]	≤20 g per day[a]

[a] Recommendations for daily total prebiotic intake and resistant starch reported by Douglas and Sanders (2008).
[b] Recommendations for daily galactooligosaccharide intake derived from Carabin and Flamm, (1999) and Silk et al. (2009).

and verbascose concentrations increasing; resistant starch may either increase or decrease after cooking. This opens up interesting lines of inquiry including how heating is related to saccharide degradation and synthesis, and if prebiotic efficacy of different fructan constituents varies. Lentils are also consumed as germinated seeds, which Vidal-Valverde and Frias (1992)reported to contain reduced concentrations of RFO. Concentrations of other prebiotic compounds throughout germination have not been studied. Finally, prebiotic compounds may function differently depending on the associated food matrix, requiring bio-efficacy studies to determine actual microbiotal and physiological effects of these compounds when consumed as a constituent of lentil.

5. Conclusions

Prebiotic carbohydrates are important component of healthy diet, supporting beneficial hindgut microflora. Total prebiotic carbohydrate concentrations suggest that a 100 g serving of lentils may provide over 13 g of prebiotics. In conclusion, our study results clearly show that lentils contain nutritionally significant amounts of prebiotic carbohydrates and, that it may be possible to enhance those amounts through breeding and locational sourcing.

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