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Original Research Article

Processing, cooking, and cooling affect prebiotic concentrations in lentil (*Lens culinaris* Medikus)

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ABSTRACT

Lentil is an important staple food crop in many regions and is a good source of protein and various micronutrients. Lentil also contains raffinose family oligosaccharides (RFO), resistant starch (RS), and other prebiotic compounds essential for maintenance of healthy gastrointestinal microflora. However, there is a lack of information about concentrations of prebiotics in commercially available, cooked, and processed lentil market classes. This study assessed concentrations of RFO and RS in two commercially available lentil market classes (medium green and small red) and determined changes associated with dehulling, cooking, cooling, and reheating. Mean total RFO concentrations ranged from 5.5 to 6.1% in raw lentils. Total RFO concentration decreased from raw to reheated seeds in two of the four lentil products: whole red (6.1–4.9%) and whole green (5.5–4.3%). Mean RS concentrations in raw, cooked, cooled, and reheated lentil (3.0, 3.0, 5.1, and 5.1% (dry weight), respectively) clearly demonstrate cooling-induced synthesis of RS from gelatinized starch. These results highlight the impact and importance of processing techniques on lentil nutritional quality for both consumer and food industry use.

1. Introduction

To better understand the relationship between diet and disease, many researchers have focused on health-beneficial bioactive components present in commonly eaten foods. Prebiotics are an important group of food constituents with positive implications for human health, including reducing risk factors for non-communicable diseases via interactions with the hindgut microbiome (Gibson and Roberfroid, 1995). According to a revised definition by Roberfroid (2007), a prebiotic is 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. Except in the case of sugar alcohols, prebiotic carbohydrates fall under the category of dietary fiber (IOM, 2005). Malabsorption of these fibers in the upper digestive tract contributes to the low glycemic response characteristic of many prebiotic-rich foods (Jenkins et al., 1981). Commonly eaten foods that contain high concentrations of prebiotics include Jerusalem artichoke (*Helianthus tuberosus* L), chicory (*Cichorium intybus* L.), garlic (*Allium sativum* L.), onion (*Allium cepa* L.), and lentil (*Lens culinaris* Medikus) (van Loo et al., 1995; Johnson et al., 2013).

Lentil is a cool-season food legume grown in many parts of the world with Mediterranean or cooler weather conditions. Being a good source of protein and a means of fixing atmospheric nitrogen, promoting sustainable agriculture, lentil is an integral component of many food systems. Current world lentil production is approximately 4.6 million metric tons (FAOSTAT, 2012). Lentil is well-suited to growing conditions in western Canada (responsible for approximately 30% of global production) and is also an emerging specialty crop in both the Pacific Northwest and the Midwest of the United States. Lentil grown in North Dakota is composed of 8.3% moisture, 24.9% protein, 2.8% ash, and 51.9% starch (Thavarajah and Thavarajah, 2012). In addition, lentil is a good source of mineral micronutrients; a one cup serving can provide 4.3–5.3 mg of iron, 1.9–3.3 mg of zinc, and 25–401 μg of selenium (Thavarajah et al., 2008, 2009). Moreover, several

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prebiotic fibers are found in lentil including raffinose family oligosaccharides (RFO) and resistant starch (RS) (Bhatty, 1988; Wang et al., 2009). Johnson et al. (2013) estimated that approximately 7.7 g of prebiotics are contained in one cup of lentil; however, to our knowledge, there are no comprehensive reports of the concentration of prebiotics in cooked lentil.

Lentil induces a low-glycemic response (Jenkins et al., 1981), which has been attributed to lentil starch's high resistance to hydrolysis. High concentrations of RS in lentil relative to other crops is a function of many contributing factors: intact tissues and cells, a high amylose concentration (20–40% of total starch), a high soluble fiber content, the presence of antinutrients, and strong interactions between amylose chains (Hoover and Sosulski, 1985; Wursch et al., 1986; Tovar et al., 1991; Hoover and Vasanthan, 1994; Siddhuraju and Becker, 2001; Piecyk et al., 2012;). Concentrations of RS in raw and cooked lentils range from 1.6 to 8.4% (dry weight) and from 1.6 to 9.1% (dry weight), respectively (de Almeida Costa et al., 2006; Murphy et al., 2008; Wang et al., 2009; Johnson et al., 2013). Those values are higher than concentrations of RS in other raw food legumes such as moth bean (*Vigna aconitifolia* Jacq. (Marechal; 1.2%)), horse gram [*Macrotyloma uniflorum* Lam. (Verdc.), previously *Dolichos biflorus*; 2.6%], and black gram (*Vigna mungo* L.; 1.9%) (Bravo et al., 1998). In short, lentil contains significant concentrations of RFO and RS compared to other staple food crops and is a globally important source of nutrients (Johnson et al., 2013).

RFO have been considered antinutrients because of their involvement in gastrointestinal discomfort and flatulence (Fleming, 1981). Conventional plant breeding programs have aimed to reduce the RFO concentration in seeds (Frias et al., 1999); however, current opinion of RFO in staple food crops has changed (Martinez-Villaluenga et al., 2005). Regular consumption of RFO may be an important dietary tool in the prevention of chronic diseases (Cani et al., 2009; Parnell et al., 2012) in addition to providing other health benefits such as immunostimulation (Lee and Mazmanian, 2010), pathogen elimination (Manning and Gibson, 2004; Caselato et al., 2011), and stimulation of mineral uptake and deposition (Coudray and Fairweather-Tait, 1998; Yeung et al., 2005). However, there is a lack of published data with respect to the concentrations of prebiotics in commercially available, cooked, and processed lentil market classes. A basic understanding of the changes in RFO and RS concentrations during processing and cooking is important to design detailed nutritional experiments and the variability in species. Thus, the objectives of this study were to (1) assess concentrations of RFO and RS in two commercially available lentil market classes (red and green), and (2) determine changes in RFO and RS concentration associated with common processing procedures: dehulling, cooking, cooling, and reheating.

2. Materials and methods

2.1. Materials

Raffinose, stachyose, and verbascose standards (99% purity), high-purity ACS grade solvents, reagents, and enzymes were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and VWR International (Radnor, PA, USA). Lentil cultivar CDC Redberry (Saskatoon, SK, Canada) and regular corn starch (Megazyme International Ireland Ltd., Wicklow, Ireland) were used as laboratory reference materials. Water was distilled and deionized (ddH_2O) to a resistance of 18.2 MΩ (Milli-Q Water System, Millipore, Milford, MA, USA) for sample extractions and preparation.

2.2. Lentil seed samples

Bulk processed lentil seed samples (2 kg) were collected from United Pulse Trading, Inc (Williston, ND, USA). Seeds of two commercially available market classes were selected based on local and international consumer preference: small red (CDC Redberry) and medium green (CDC Richlea). Selected small red lentil samples included (1) whole seed with an intact seed coat and (2) split and decorticated as generally marketed for local and international markets. For medium green lentils, selected samples were (1) whole seed with an intact seed coat and (2) decorticated. Bulk lentil samples were homogenized, subsampled ($n = 4$), and stored at −60 °C prior to cooking. Additional subsamples ($n = 4$) of each bulk sample were ground to a particle size of ≤1.0 mm and stored for a short period at −60 °C until analysis of RS. The treatment design was a completely randomized design with three replicates of four commercially available lentil products (small red, whole; small red, split/decorticated; green, whole; green, decorticated), and four processing methods (raw, cooked, cooled, reheated) ($n = 48$). This entire experiment was repeated twice, therefore total of 96 samples were analyzed.

2.3. Cooking procedure

Approximately 12 g of unground seeds were placed in distilled water at a ratio of 1:3 (dry weight) in a 50 mL round-bottom test tube. Samples were suspended in a boiling water bath and cooked for 40 min. After cooking, samples were cooled to 4 °C and stored for 24 h in a refrigerator. Excess water retained after cooking was not discarded. Cooled samples were then heated to boiling in a 1300 W microwave oven (Panasonic Electric, Washington, DC, USA) on high for 60 s. Cooked, cooled and reheated lentil samples were then freeze-dried in a VirTis Sentry freeze-dryer (SP Scientific, Gardiner, NY, USA) and hand-ground to a fine powder using a mortar and pestle to measure RFO concentration. Moisture content ($n = 96$) for each sample was determined gravimetrically (AACC, 2000).

2.4. Determination of RFO concentration

Freeze-dried finely ground samples (0.5 g) were incubated with ddH_2O for 1 h at 80 °C to extract RFO, as previously described (Muir et al., 2009). After centrifugation at 3000 *g* (GPR Centrifuge, Beckman Coulter Inc., Brea, CA, USA), a 1.0 mL aliquot of the supernatant was passed through a 13 mm × 0.45 μm nylon syringe filter (Chromatographic Specialties, Brockville, ON, Canada). Oligosaccharide analysis was conducted using a previously published method (Feinberg et al., 2009), modified for optimal peak separation. Chemical separation and analysis of RFO was performed on a Dionex system (ICS-5000 Dionex, Sunnyvale, CA, USA). RFO were separated using a CarboPac PA1 column (250 mm × 4 mm; Dionex, Sunnyvale, CA, USA) in series with a CarboPac PA1 guard column (50 mm × 4 mm; Dionex, Sunnyvale, CA, USA). 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). Sample analysis began with a linear gradient change at a flow rate of 1 mL/min from 50% solvent B and 50% solvent C to 0.5% A, 49% B, and 49% C at 3 min. Subsequent gradient changes altered solvent concentrations to 3% A, 47% B, and 47% C by 16 min and to 16% A, 42% B, and 42% C by 18 min. The final interval resumed initial conditions of 50% B and 50% C for a total run time of 20 min. Detection of RFO was carried out using a pulsed amperometric detector with a working gold electrode and a silver-silver chloride electrode at 2.0 μA (Dionex, Sunnyvale, CA, USA). The system identified and quantified RFO concentrations based on pure standards (>99%). Concentrations of RFO were detected within a linear range of 3–100 μg/g, with a minimum detection limit of 0.2 μg/g. An external lab reference, CDC Redberry, was also used daily to ensure