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Carbohydrate composition of mature and immature faba bean seeds

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ABSTRACT

Faba bean is a valuable pulse crop for human consumption. The low molecular weight carbohydrates (LMWC): glucose, fructose, sucrose (GFS), raffinose, stachyose, and verbascose (RFO- raffinose family oligosaccharides) in faba bean seeds contribute to the flavor and prebiotic nature of this edible bean. Understanding the variation of these compounds across the species would aid plant breeders in their selection efforts to release improved varieties. Therefore, this study was conducted to quantify LMWCs from a diverse collection of faba bean germplasm. The LMWCs of mature and immature seed from 40 faba bean populations across a range of seed sizes (26.2–172.0 g 100 seed^{-1}) were quantified with an Agilent 1260 Infinity LC (size exclusion chromatography/gel permeation chromatography) system with refractive index detection. Sucrose was the predominant constituent LMWC of immature seeds ranging from 5.9 to 22.6% DW for cotyledons and 6.7 to 16.7% DW for seed coats, while total RFO averaged <1% DW across populations. The sucrose content of mature seeds was relatively stable across population with a mean of 2.4% DW, while RFO content ranged from 2.5 to 7.5% DW. The apparent positive relationship between seed size and GFS of immature seed and sucrose and RFO of mature seed indicates that selection for seed size may also affect LMWC concentration.

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1. Introduction

Faba bean (*Vicia faba* L.) is a versatile cool-season pulse crop utilized for human and animal consumption (Crépon et al., 2010). There are three main botanical varieties of faba bean: var. minor (<40 g 100 seed^{-1}), var. equina (40–80 g 100 seed^{-1}), and var. major (>80 g 100 seed^{-1}) (Duc, 1997). Minor and equina types are mainly utilized as mature dry seeds, while immature seed of var. major is most often consumed as a fresh vegetable (Hawtin and Hebblethwaite, 1983; Baginsky et al., 2013).

The main energy components and therefor value of mature faba bean seed is most often attributed to starch (~40% DW) and protein (~30% DW) contents (Pritchard et al., 1973; Guillon and Champ, 2002). However, low molecular weight carbohydrates (LMWC): glucose, fructose, sucrose (GFS) and raffinose family oligosaccharides (RFO): raffinose, stachyose, and verbascose also contribute to seed quality (White, 1966; El-Shimi et al., 1980; Freijnagel et al., 1997; Duc et al., 1999).

The majority of interest has been given to RFOs as they are presumed to be antinutritional (Frauen et al., 1984; Ruperez, 1998) or non-nutritional (Torres et al., 2012). However, alternative opinions suggest that these flatulence-causing carbohydrates may also serve as important health promoting prebiotics (Tomomatsu, 1994; Frias et al., 1996; Champ, 2002; Martinez-Villaluenga et al., 2008). Hayakawa et al. (1990) showed that low doses (3 g day^{-1}) of purified RFO could increase bifidobacteria and Kozlowska (2001) suggested 0.1–0.3 g of RFO per portion would not likely cause digestive issues.

Sucrose is another LMWC considered to be a critical component of many foods, as it enhances natural flavors and improves the characteristics of other flavoring ingredients (Grotz and Munro, 2009). For example, genotypes of dry mature cowpeas with a sucrose content of 6% compared to 2% were consistently chosen for flavor (Hall et al., 2003). Furthermore, small additions of 5% and 10% sucrose to the fiber rich butterbean (*Phaseolus lunatus* L.) enhanced taste and acceptance (Vorster et al., 1987).

The monosaccharides fructose and glucose are generally negligible in mature faba bean seed, but sucrose content has a wide distribution and can range from 0.02% to 5.23% DW (Pritchard et al., 1973; Cerning et al., 1975; Frauen et al., 1984; Lattanzio et al., 1986; Quemener, 1988; Frias et al., 1996). Comparatively less information is available for the LMWC content of immature faba bean seeds (Lattanzio et al., 1986; Ziena et al., 1987; Frias et al., 1996; Weber et al., 1996) especially from a diverse panel of germplasm.

Screening faba bean germplasm for LMWC content will help to inform breeders in their efforts to improve the sweetness and perhaps flavor of mature and immature seeds, while minimizing

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RFO content. Identification of genotypes with these desirable seed quality traits would help to expand market potential. The purpose of this research was to quantify LMWCs from 40 faba bean populations varying in seed size. Large to small-seeded classes obtained from commercial sources and germplasm accessions served to broadly characterize LMWCs of faba bean.

2. Materials and methods

Forty faba bean populations (commercially available cultivars, open pollinated heirlooms, germplasm accessions from the USDA-ARS Western Regional Plant Introduction Station, and a breeding line 'HE-F_5', derived from a cross between winter-hardy European cultivar 'Hiverna/2' and a large-seeded commercial cultivar 'Extra Precoce Violetto' at the F_5 generation) were included in this study (Supplemental Table 1). A representative sample of air dried mature seeds and immature seeds were selected from field grown plants in 2013 at Washington State University's Whitlow Farm (WF) in Pullman, WA (46°44′3.2″N–117°7′25.8″W) and dehulled.

Immature seeds were harvested during the late cotyledon stage from 30 to 50 days after flowering when the sucrose content is reported to be highest (Lattanzio et al., 1986; Heim et al., 1993; Borisjuk et al., 1995; Frias et al., 1996). Collected immature cotyledons were separated from the seed coat and percent moisture was determined once lyophilized. Mature seeds were dehulled and the hulls discarded, since they contained negligible amount of LMWC (data not shown).

LMWC extraction and analysis was followed according to Knudsen and Li (1991) using water as the mobile phase instead of 0.015 N Na_2SO_4. Briefly, triplicate 200 mg samples of finely ground flour were extracted with 4 mL of EtOH (50%, v/v) plus 1 mL of ribitol as an internal standard for 1 h at room temperature with intermittent shaking. The supernatant was then decanted after centrifugation (3000*g*, 10 min @ 24 °C) and the pellet was rinsed with 3 mL and then 2 mL of EtOH (50%, v/v) after a 5 min reflux cycle. The supernatants were pooled and diluted to 10 mL with EtOH (50%, v/v). An aliquot (1.0 mL) from this stock was diluted with an equal volume of EtOH (90%, v/v), stored at −20 °C for 1 h and centrifuged (10,000*g* for 10 min @ 24 °C) to precipitate proteins. The supernatant was dried at 25 °C (Thermo Scientific DNA 110 Savant™ SpeedVac™, Waltham, MA, U.S.A.) and redissolved in 1 mL of ultrapure water (EMD Milipore Co.; Billerica, MA, U.S.A.) for high performance liquid chromatography (HPLC) analysis.

An Agilent 1260 Infinity LC system (size exclusion chromatography/gel permeation chromatography) with refractive index detection (Agilent Technologies, Santa Clara, CA, U.S.A.) at 35 °C was used to quantify individual LMWCs. The mobile phase was water with a flow rate of 0.5 mL min^{-1}. An Aminex HPX-N87 (300 × 7.8 mm) resin-based column (Bio-Rad Laboratories, Inc.; Hercules, CA, U.S.A.) in the sodium form (300 × 7.8 mm) preceded by a Cation H Bio-Rad Micro-Guard column (304.6 mm) was used to separate LMWCs isocratically at 80 °C. Injection volume of the sample was 20 μL and run time was 15 min.

Quantification and assignment of peaks was based on the peak areas and retention times of known standard curves for purified glucose (C.A.S. 50-99-7, purity ≥99.5%), sucrose (C.A.S. 57-50-1, purity ≥99.5%), fructose (C.A.S. 54-48-7, purity ≥99%), verbascose (C.A.S. 546-62-3, purity ≥ 97%), stachyose (C.A.S 54261-98-2, purity ≥98%), raffinose (C.A.S. 17629-30-0, purity ≥98%), and ribitol (C.A.S. 488-81-3, purity ≥99%) (Supplemental Fig. 1) (Sigma-Aldrich Co.; St. Louis, MO, U.S.A.). Linearity of analysis was assessed based on standard curves at concentrations between 80 and 120% of the working range in sample concentrations. The minimal detectable limit across analytes was between 0.05 and 0.1 ppm. The coefficient of determination between peak area (y) and the mass in mg of glucose (0.999), sucrose (0.9998), fructose (0.9997), stachyose (0.9998), raffinose (0.9998), and verbascose (0.9999) was high. Precision expressed as the percentage of relative standard deviation at each concentration for each analyte was <4% based on three injections (n = 3) (data not shown). Accuracy and reproducibility of detection was below 5% based on independent runs. All samples were chromatographed under the same conditions as the standards and presented on a percent dry weight (% DW) basis.

LMWC contents of mature and immature seeds were compiled and analyzed ($p \leq 0.05$) using an ANOVA and Tukey's honest significant difference test with PROC MIXED (SAS, 2008). Analyses were conducted according to a completely randomized design with three biological replications and standard error bars are presented. Significant entry differences were assessed once significant seed type (mature seed, immature seed coat, and immature cotyledon) and seed weight class (small: <41 g 100 $seed^{-1}$, medium: 42–85 g 100 $seeds^{-1}$, and large: >85 g 100 $seeds^{-1}$) category differences were identified.

3. Results and discussion

The current HPLC protocol worked very well to quantify individual LMWCs of faba bean seeds. The use of water as a mobile phase had the benefit of eliminating toxic waste disposal and provided simplicity and speed, however in this study it occasionally failed to fully resolve baselines in the mature seed for stachyose and verbascose (Supplemental Fig. 2). The retention time for each sugar was in most cases the same as that of the standard, but occasionally varied (0.2–0.4 s) for fructose and constituent RFOs.

The LMWC elution patterns (Supplemental Fig. 2) and dry weight concentrations (Table 1) were characteristic for each sample tissue. This was expected based on the invertase control hypothesis of legume seed development (Weber et al., 1997); where cell wall invertase activity within the seed coat is related to growth and a high hexose state, whereas increasing sucrose synthase activity marks the transition from growth to storage product synthesis and maturation. The accumulation of RFOs and concomitant decline in sucrose concentration marks this transition from dry weight accumulation to desiccation and a mature quiescent state (Obendorf and Górecki, 2012).

As expected, RFOs and sucrose predominated in mature seed, while sucrose constituted the major LMWC of immature seed. The primary RFOs of mature seed averaged across populations were verbascose (2.4% DW) and stachyose (1.9% DW), in agreement with past reports (Lattanzio et al., 1986; Quemener, 1988; Dini et al., 1989). Sucrose was highest for immature cotyledon (13.4% DW) and seed coat (8.0% DW) tissues and lowest for mature seed (2.5% DW). In general, the sucrose content of the var. major immature (15.7% DW) and mature (3.0% DW) cotyledons were 20% to 30% higher than either of the var. equina or var. minor classes. These results are consistent with others that have reported larger seeds generally have higher sucrose values than genotypes with smaller seeds (Cerning et al., 1975; Barratt, 1992; Lattanzio et al., 1986).

For simplicity total glucose, fructose, and sucrose (GFS) from immature seed (Fig. 1) and sucrose and raffinose, verbascose, and stachyose (RFOs) contents from mature seed (Fig. 2) are presented for individual populations because of low concentrations (0.2% DW) in other tissues. The wider distribution for GFS content of immature seed (Fig. 1) as compared to sucrose or RFOs of mature seed (Fig. 2) across individual populations may be, at least in part, the result of variable seed maturation shown as moisture content of harvested immature seed (Supplemental Table 1). However, previous reports have also documented that GFS can range from 7% to 15% DW for whole immature faba bean seeds across populations