Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 72 (2008) 664–672
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Varietal differences of carbohydrates in defatted soybean flour and soy protein isolate by-products

E.M. Bainy [a,b], S.M. Tosh [a,*], M. Corredig [b], V. Poysa [c], L. Woodrow [c]

[a] *Food Research Program, Agriculture and Agri-Food Canada, Guelph, Ont., Canada N1G 5C9*
[b] *Department of Food Science, University of Guelph, Guelph, Ont., Canada N1G 2W1*
[c] *Harrow Research Centre, Agriculture and Agri-Food Canada, Harrow, Ont., Canada N0R 1G0*

Received 19 June 2007; received in revised form 6 October 2007; accepted 11 October 2007
Available online 17 October 2007

Abstract

Soy protein isolates (SPI) were prepared from 12 soybean lines grown in Harrow, Ontario and by-products (fibers and wheys) from SPI making were saved. The identification and quantification of soluble sugars in defatted flours, fibers and wheys were carried out using high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) and with a colorimetric method for uronic acids. Defatted flours and fibers were acid hydrolyzed, then analyzed by HPAEC-PAD for monosaccharide composition. The results showed varietal differences in the carbohydrate composition suggesting different applications for these defatted flours and their SPI by-products.
Crown copyright © 2007 Published by Elsevier Ltd. All rights reserved.

Keywords: Soybeans; Soy protein isolates; Carbohydrates; Polysaccharides; Oligosaccharides; Sucrose

1. Introduction

Defatted soybean flour consists of approximately 50% protein, 40% carbohydrate and other minor components. Therefore, soy carbohydrate is the second largest component after protein indicating an important economical value for the soy-food industry (Aspinall, 1988). The carbohydrate fraction includes simple sugars (mono- and disaccharides), oligosaccharides and polysaccharides – mainly cellulose, hemicellulose, pectin and starch (Liu, 1999).

Over 99% of sugars in mature soybean seed are sucrose and the oligosaccharides raffinose and stachyose (Hymowitz, Walker, Collins, & Panczner, 1972a). Sucrose is in the range of 2.5–8.2%, raffinose may vary from 0.1% to 0.9%, and stachyose 1.4% to 4.1%, of the seed dry weight, depending on cultivars and growing environments (Hymowitz, Walker, Collins, & Panczner, 1972b).

High sucrose content is a desirable component responsible for the taste of soy products (Taira, 1990). Soybean oligosaccharides, raffinose and stachyose, which resist digestion due to the α-galactoside linkages in their structure, are responsible for flatulence and abdominal discomfort when ingesting soybean products (Rackis, 1975). These soybean oligosaccharides have prebiotic effects, however, and studies have shown that their consumption is related to several health benefits, such as lowering blood cholesterol, reducing blood pressure and preventing some types of cancer (Roberfroid, 2007; Tomomatsu, 1994).

In the whole soybean, polysaccharides can be separated into major non-cellulosic and minor cellulosic internal cell wall structural parts (Aspinall, 1988). The non-cellulosic fraction consists of acidic polysaccharides, arabinogalactan and arabinan chains (Slavin, 1988). Acidic polysaccharides have D-galacturonic acid and L-rhamnose in the main chain with side chains consisting of mainly galactose and arabinose residues (Schols & Voragen, 1998). The consumption of these non-digestible polysaccharides seems to be related to physiological effects such as improving gastrointestinal

* Corresponding author. Tel.: +1 519 780 8019; fax: +1 519 829 2600.
E-mail address: ToshS@agr.gc.ca (S.M. Tosh).

0144-8617/$ - see front matter Crown copyright © 2007 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2007.10.008

functions, glucose tolerance (Tsai et al., 1983) and cholesterol-lowering ability (Carrol, 1991).

In this paper, 12 Ontario grown soybean lines were studied. The parent line, Harovinton, and its 11 derived lines have high protein content (47–52%) with varying amounts of specific 7S and 11S protein subunits. The new lines were conventionally bred with the objective of altering the protein subunit profiles. Some studies focused on the differences in protein composition of these soybean lines have been reported (Poysa & Woodrow, 2002; Poysa, Woodrow, & Yu, 2006; Zarkadas et al., 2007). However, it is also important to understand whether changes in protein composition are accompanied by changes in the carbohydrate fraction of soybeans, especially in light of possible new uses of the carbohydrate fractions because of their nutritional functionality.

Soy protein isolate (SPI) was prepared from defatted soybean flour. When making SPI, carbohydrates must be removed producing carbohydrate-rich residues that may have useful applications. These residues, referred to in this study as fiber and whey, are rich in simple sugars, oligosaccharides and polysaccharides. The objectives of this study were to explore varietal differences and evaluate the types of by-products generated during SPI production from the parent line and 11 modified soybean lines. Soluble sugars, uronic acids and monosaccharide composition are reported for these lines.

2. Materials and methods

2.1. Materials

The parent variety, Harovinton, and its 11 derived soybean lines were grown in 2005 in Harrow, Ontario at the Greenhouse and Processing Crops Research Centre of Agriculture and Agri-Food Canada. Cultivar names and protein composition of the 12 soybean lines are described in Table 1. The soybean flour was defatted using hexane extraction at 23 °C. The defatted flour was the starting material to prepare soy protein isolated from the 12 soybean lines. The two main residues, fiber and whey, from the soy protein isolation were collected and freeze dried. The proximate analysis of the defatted flour, fiber and whey were determined.

Moisture content was determined by drying the samples in an oven at 100 °C until no further change in weight. Protein content was determined by Dumas combustion method (Leco FP-528 Mississauga, ON). The conversion factor used was $N \times 6.25$. Ash content was determined by charring the samples in an oven at 500 °C overnight.

2.2. Preparation of soy protein isolate

SPI was prepared from flours defatted at room temperature to prevent heat denaturation of the proteins, according to previous literature (Renkema, Lakemond, de Jongh, Gruppen, & van Vliet, 2000) with slight modifications as outlined in Fig. 1. The flour was suspended in 100 mM Tris–HCl buffer at pH 8.0 in a 1:10 ratio (w/v), and stirred at room temperature for 1 h. Fiber was separated by centrifugation (12,000*g*, 30 min, 10 °C) using a Beckman Coulter Model J2-21 (Follerton, CA) and recovered using porcelain filter with a filter paper (Fisher Brand Qualitative P8 Filter Paper, Fisher Scientific, Pittsburgh, PA). The supernatant was adjusted to pH 4.8 with 2 M HCl to induce precipitation of soy proteins. After 2 h at 4 °C the dispersion was centrifuged as described above. The soluble phase from this centrifugation step (whey) was collected for further analysis. The precipitate was washed with 10 mM sodium acetate buffer at pH 4.8 (1:8 ratio (w/v)) and centrifuged as described above and the supernatant from this washing step was discarded.

The final precipitate (SPI) was suspended in MilliQ water, adjusted to pH 7.5 and dialyzed overnight. SPI, fiber and whey were freeze dried.

2.3. Ethanol extraction procedure

Low molecular weight sugars were extracted from defatted flour and fiber samples by refluxing in 70% ethanol at a solvent-to-sample ratio of 10:1 with constant stirring for

Table 1
Protein subunit profile of 12 Ontario soybean lines

Name	Soybean genotypes Cultivar names	Protein composition Subunits	
	Genotype designation	Absent subunits	Present subunits
Harovinton[a]	Harovinton	–	–
Line 2	SQ98-0110-3-1	A3	α, α′, β, A1A2, A4
Line 3	SQ97-0263-54-1-5	α′, A4	α, β, A1A2, A3
Line 4	SQ98-0105-6-1	α′, A3	α, β, A1A2, A4
Line 5	SQ97-0263-71-1-3	A1A2, A4	α, α′, β, A3
Line 6	SQ98-0105-1-1b	A3, A4	α, α′, β, A1A2
Line 7	SQ97-0263_21-7-2	α′, A3, A4	α, β, A1A2
Line 8	SQ97-0263_3-10-1	α′, A1A2, A3	α, β, A4
Line 9	SQ970252_S17-2-1	A1A2, A3, A4	α, α′, β
Line 10	SQ970252_S17-2-3	A1A2, A3, A4	α, α′, β
Line 11	SQ97-0263_3-1a	α′, A1A2, A3, A4	α, β
Line 12	SQ98-0112-S7-1	A1A2	α, α′, β, A3, A4

[a] Parent variety.

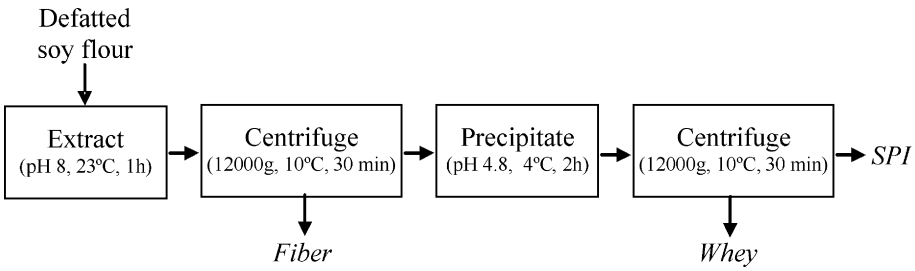

Fig. 1. Procedure 1 for producing soy protein isolate and its by-products from defatted soybean flour.

2 h at 90 °C. The extracted residue was separated by centrifugation (10,000*g*, 20 min, 20 °C) using a GS-15R centrifuge (Beckman Coulter Canada Inc., Mississauga, ON), and then washed first with 70% ethanol, recentrifuged (as above) and then with 95% ethanol. After the third centrifugation step, the washed defatted flour and fiber (with soluble sugars removed) were collected for further analysis. The supernatant of all the washes extracted from the defatted flour and fiber was evaporated to dryness using a rotavapor (EL130, Buchi, Flawil, Schweiz).

2.4. Quantification of soluble sugars

The soluble sugar composition of the defatted flour, fiber and whey fractions was studied. Recent studies have demonstrated that the use of high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) for sugar analysis results in highly selective and sensitive determination of the sugar components (Giannoccaro, Wang, & Chen, 2006; Schols & Voragen, 1998). Dried supernatants from the alcohol extraction of defatted flour and fiber and freeze dried whey fractions were resuspended in appropriate amounts of MilliQ water and soluble sugars were determined using HPAEC-PAD (Dionex D × 500, Sunnyvale, CA) with a CarboPac PA1 column (4 × 250 mm) and guard (3 × 25 mm). Samples were filtered (0.45 μm) prior to analysis and injected with an AS40 automated sampler (Dionex). The column was washed for 15 min before each injection with 0.3 M NaOH. The sample was then eluted for 25 min at 10 mM NaOH followed by 20 min at 0.1 M NaOH. The flow rate was 1.0 mL/min and the oven temperature was kept at 35 °C. Pulse potentials (E, volts) and durations (t, ms) were $E_1 = 0.05$, $t_1 = 480$, $E_2 = 0.6$, $t_2 = 180$; $E_2 = 0.6$, $t_2 = 180$, $E_3 = -0.6$, $t_3 = 60$ with a 1.0 s detector response time.

Standard curves were prepared using sucrose, raffinose and stachyose at six different concentrations ranging from 10 to 150 μg/mL for each sugar. Standards were also used to identify the different sugars based on their retention times. Sugar standards were included with every group of samples loaded to the system. All sugar analyses were performed in triplicate and results were expressed on a dry-weight percentage basis. The total amount of soluble sugars was based on chromatogram analyses carried out with Chromeleon version 6.50 software (Dionex).

2.5. Uronic acids

The uronic acid content was determined by the colorimetric *m*-hydroxydiphenyl assay (Blumenkrantz & Asboe-Hansen, 1973). Absorbance was measured at 520 nm using a Cary 300 UV–vis Spectrophotometer (Varian Inc., Mississauga, ON). A standard curve was constructed with galacturonic acid monohydrate (97% pure, Aldrich Chemical Co., Milwaukee, WI) as standard.

2.6. Sugar composition of the polysaccharide fractions

Selected lines were also analyzed further for sugar composition of their insoluble carbohydrate fractions. Selected defatted flour and washed fiber (soluble sugars removed) were acid hydrolysed to their monosaccharide subunits with sulphuric acid. Samples, approximately 10 mg, were weighed into screw capped test tubes and 1 mL of 1 M H_2SO_4 was added. The tubes were placed in a glycerol bath with constant stirring at 100 °C for 3 h. After cooling to room temperature, the hydrolysates were diluted, filtered through a 0.45 μm filter, and then analyzed by HPAEC-PAD as described above. Samples were eluted with 8 mM NaOH for 7 min, followed by 28 min elution with MilliQ water. Standard curves were prepared with rhamnose, arabinose, galactose, glucose, xylose and mannose with concentrations from 5 to 100 μg/mL. All analyses were performed in triplicate and expressed on a dry-weight percentage basis.

2.7. Statistical analysis

Statistical evaluation was conducted by using General Linear Model (GLM) and Least Squares Means (LSMEANS) procedures with SAS (version 8.0, Cary, NC) to determine significant differences among the 12 soybean lines.

3. Results and discussion

When isolating soybean proteins, carbohydrates have to be extracted from the defatted flour to subsequently yield a

high protein isolate (SPI). The carbohydrates removed represent approximately 20–30% waste during SPI making. Defatted flours and the two main by-products of SPI, fiber and whey, of 12 soybean lines were studied for soluble sugar composition using a HPAEC-PAD system. Selected lines were also further studied for the monosaccharide composition of the insoluble fractions with the HPAEC-PAD. Fig. 2 shows a representative elution pattern in HPAEC-PAD for a defatted flour sample of Harovinton. It was possible to clearly identify six monosaccharide peaks: rhamnose, arabinose, galactose, glucose, xylose and mannose.

The composition of the defatted flour from the 12 soybean lines studied is summarized in Table 2. The results showed significant differences ($P < 0.0001$) among the soluble sugar contents of the 12 soybean lines in defatted flour, fiber and whey fractions. Five lines showed similar total soluble sugar content with an average of 10.2%, three lines showed lower sugar contents with an average of 8.5% and four lines showed a higher sugar content with an average of 11.6%. Line 4 had the lowest and line 11 the highest total soluble sugar content. In addition, differences were observed in the proportion of total soluble sugars in the total carbohydrates (calculated by difference); for instance, in lines 3 and 4 soluble sugars made up 19% of the total carbohydrate compared to a value of 26% for lines 6 and 11. The differences were also confirmed when comparing specifically the total sucrose and total oligosaccharides as a percentage of total carbohydrates. In the other lines had a soluble carbohydrates represent 23% of total carbohydrates.

Fig. 3 illustrates the soluble carbohydrate content of the defatted flour as well as the corresponding whey fraction. Lines 3 and 4 had the lowest (3.7%) and lines 10 and 12 had the highest (5.5%) sucrose content. High sucrose soybean lines may find possible uses to improve flavour and digestibility of soybean products. In addition, soluble sugars are important for fermentation processes such as those typical for production of natto and miso. Lines 3 and 4 also presented the lowest raffinose and stachyose contents (4.5%) compared to the other lines. A high concentration of raffinose and stachyose (6.5%) was shown for lines 6,

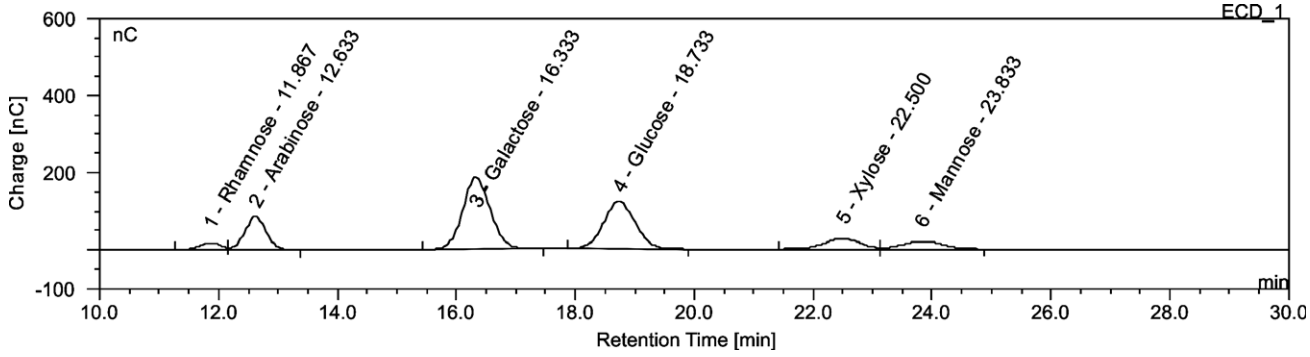

Fig. 2. Separation of sugars in acid-hydrolyzed defatted flour from Harovinton variety using high-performance anion-exchange chromatography associated with pulsed-amperometric detection 2(HPAEC-PAD). Monosaccharide rhamnose (1), arabinose (2), galactose (3), glucose (4), xylose (5) and mannose (6) are shown with their respective retention times.

Table 2
Composition of defatted flour of 12 soybean lines (percentage dry-weight basis)[A]

Variety	Protein	Ash	Total CHO[B]	Sucrose	Raffinose	Stachyose	Total soluble sugars[C]	Uronic acids (UA)
Harovinton	$51.24 \pm 0.19^{e,f}$	5.77 ± 0.10^{e}	42.99	4.72 ± 0.08^{e}	1.32 ± 0.01^{f}	3.99 ± 0.05^{d}	10.04 ± 0.14^{c}	7.96 ± 0.31^{b}
Line 2	47.02 ± 0.29^{a}	$5.52 \pm 0.02^{b-d}$	47.46	4.67 ± 0.02^{e}	1.06 ± 0.01^{e}	4.47 ± 0.04^{g}	$10.20 \pm 0.08^{c,d}$	9.11 ± 0.41^{d}
Line 3	50.63 ± 0.39^{e}	5.79 ± 0.26^{e}	43.58	3.57 ± 0.06^{a}	0.91 ± 0.00^{c}	3.72 ± 0.01^{b}	8.21 ± 0.06^{a}	$7.69 \pm 0.33^{a,b}$
Line 4	52.08 ± 0.47^{g}	6.04 ± 0.14^{f}	41.88	3.82 ± 0.02^{b}	0.78 ± 0.02^{a}	3.53 ± 0.02^{a}	8.13 ± 0.06^{a}	$7.50 \pm 0.08^{a,b}$
Line 5	48.78 ± 0.16^{c}	$5.59 \pm 0.13^{c-e}$	45.63	4.21 ± 0.01^{c}	1.23 ± 0.00^{g}	3.86 ± 0.01^{c}	9.31 ± 0.02^{b}	$7.52 \pm 0.11^{a,b}$
Line 6	49.45 ± 0.34^{d}	5.78 ± 0.03^{e}	44.77	5.20 ± 0.01^{g}	1.41 ± 0.01^{h}	5.07 ± 0.03^{j}	11.67 ± 0.05^{f}	7.87 ± 0.49^{b}
Line 7	49.46 ± 0.52^{d}	6.10 ± 0.03^{f}	44.44	5.04 ± 0.01^{f}	0.93 ± 0.00^{c}	4.19 ± 0.01^{e}	10.16 ± 0.01^{c}	$8.84 \pm 0.30^{c,d}$
Line 8	$51.47 \pm 0.28^{f,g}$	$5.59 \pm 0.10^{c-e}$	42.94	4.38 ± 0.03^{d}	0.83 ± 0.01^{b}	4.91 ± 0.03^{i}	10.12 ± 0.07^{c}	7.94 ± 0.32^{b}
Line 9	$48.27 \pm 0.53^{b,c}$	$5.39 \pm 0.06^{b,c}$	46.34	5.06 ± 0.04^{f}	0.91 ± 0.01^{c}	4.39 ± 0.02^{f}	10.36 ± 0.04^{d}	8.52 ± 0.15^{c}
Line 10	48.01 ± 0.35^{b}	$5.32 \pm 0.05^{a,b}$	46.67	5.65 ± 0.13^{i}	1.08 ± 0.02^{e}	5.15 ± 0.10^{k}	11.87 ± 0.26^{g}	8.64 ± 0.14^{c}
Line 11	49.64 ± 0.45^{d}	5.10 ± 0.05^{a}	45.27	$5.13 \pm 0.04^{f,g}$	0.99 ± 0.03^{d}	5.78 ± 0.04^{l}	11.90 ± 0.05^{g}	7.40 ± 0.13^{a}
Line 12	50.84 ± 0.21^{e}	$5.74 \pm 0.03^{d,e}$	43.42	5.43 ± 0.02^{h}	0.86 ± 0.01^{b}	4.78 ± 0.02^{h}	11.06 ± 0.03^{e}	$7.58 \pm 0.15^{a,b}$

Values are reported as mean value ± SD.
[A] Data are means of triplicate measurements.
[B] Total carbohydrates determined by difference.
[C] Sum of sucrose, raffinose and stachyose.
[a–l] Means in a column with different letters are significant different ($P < 0.05$).

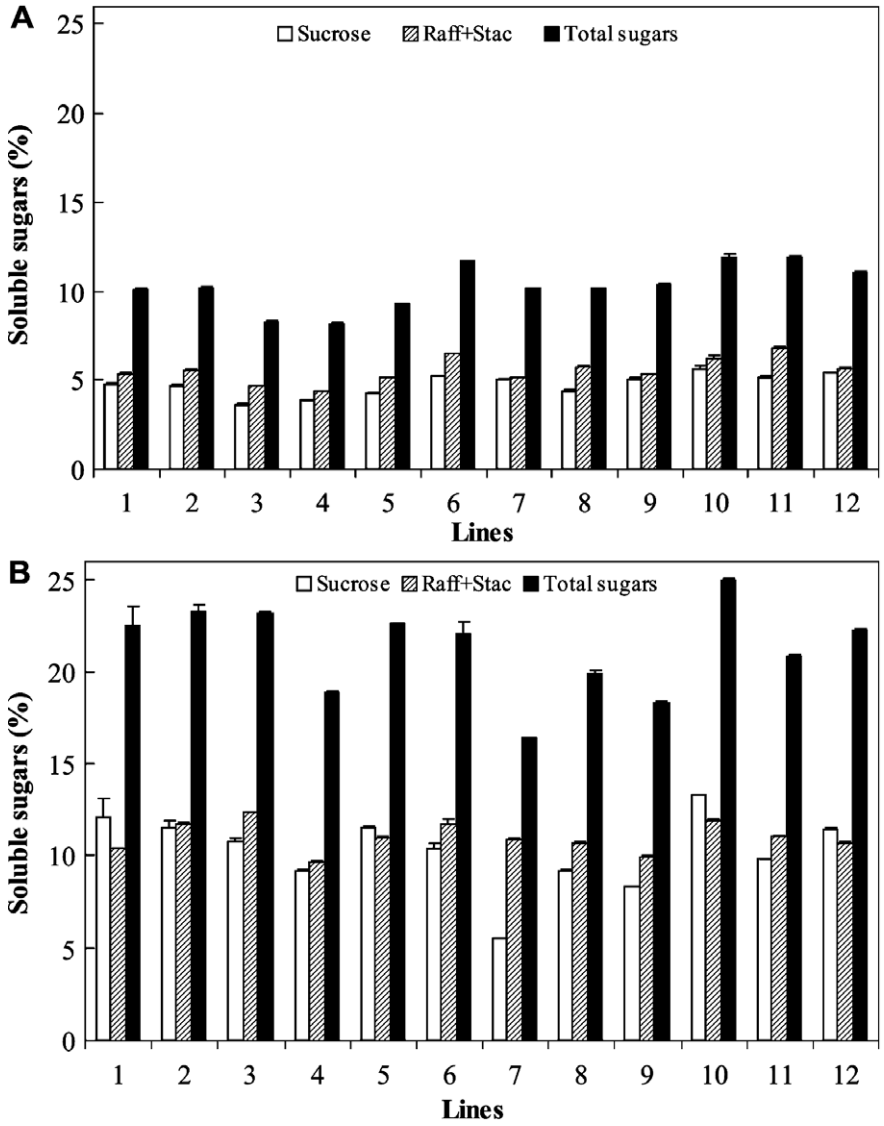

Fig. 3. Soluble sugar contents of defatted flours (A) and whey by-products (B) from 12 soybean lines. Error bars represent 95% confidence interval.

10 and 11. These oligosaccharides are partially responsible for the undesirable flatulence caused by ingestion of soybean meal (Cristofaro, Mottu, & Wuhrmann, 1974). Lines containing low amounts of these sugars may be favored for the production of some soy-food products. Lines with high levels of these oligosaccharides, however, may find applications in prebiotic products.

Fig. 4 summarizes the amount of uronic acid in defatted flour and the corresponding whey and fiber. The amount of uronic acid varied from about 7.5% for lines 4, 5, 11 and 12 to 9% for lines 2 and 7. These results are in accordance with previous studies (Mullin & Xu, 2000) reporting uronic acids contents from 4.7% to 9.5% for five soybean varieties analyzed. On average, total uronic acids were 18% of total carbohydrates, with some differences among lines. Line 7 showed the highest proportion of uronic acids (19.9%) and line 11 the lowest at 16.3%.

Complex carbohydrates have considerable importance in the textural properties preferred by Japanese miso makers (Mullin & Xu, 2000); therefore varieties with different carbohydrate contents may have distinct uses in the food industry. Consequently, six selected lines were chosen from the data shown in Table 2 for analysis of the complex carbohydrate composition of defatted flours and fibers (after the soluble sugars were removed). The parent line, Harovinton, was chosen for comparison. Other lines were selected due to their high and low protein content and their high and low sugar content. Line 2 had the lowest protein content and the highest insoluble sugar content, as well as a high amount of uronic acid in the whey and fiber fractions. Line 4 had the highest protein content, the lowest soluble sugars and high uronic acid levels recovered mostly in the fiber fraction. Line 6 showed an average protein content and high soluble sugars content. Line 10 had high soluble sugar content with a high level of raffinose and stachyose. Line 12 had high protein and soluble sugars content.

The monosaccharide composition of the defatted flour, after alcohol wash, from the six selected lines, is shown

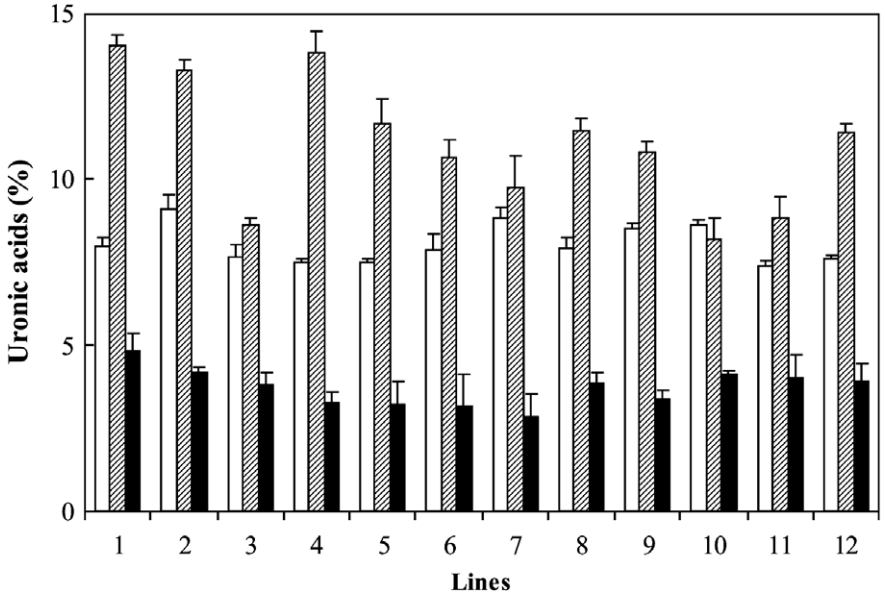

Fig. 4. Uronic acids of 12 lines of defatted flours (open bars), fibers (slashed bars) and wheys (filled bars). Error bars represent 95% confidence interval.

in Table 3. These results highlight the differences among lines in the monosaccharide composition of the insoluble fraction. Line 10 contained the most galactose (8.0%) and Harovinton and line 4 had the lowest content (6.8%). Line 2 showed the highest content of glucose (6.6%) while the other five lines averaged approximately 5.5%. The amount of xylose was not significantly different among the lines analyzed. There were significant differences in the ratio of rhamnose/arabinose: line 6 had 0.29:1 in contrast to line 4 with only 0.18:1. However, the uronic acid content was 7.7% for both lines. This may suggest that line 6 had pectins with much more branched structures than those of line 4. On the other hand, line 2 had the highest uronic acid content (9.1%) with a low ratio of rhamnose/arabinose 0.23:1 suggesting that it contains a higher content of non-branched pectins.

Table 4 summarizes the composition of the whey fractions. These fractions are rich in soluble sugars and proteins. Lines 4, 7 and 9 had the lowest concentration of soluble sugars in whey, while lines 2, 3 and 10 showed the highest amount (see also Fig. 3). Nonetheless, differences in the proportion of soluble sugars in total carbohydrates were significant among the soybean lines, confirming data reported in Table 2 for defatted flour. For example, the whey fraction from the extraction of line 10 had 0.53:1 soluble/insoluble carbohydrates, contrasting line 7 with 0.37:1. Lines 4 and 8 also showed a lower ratio (0.38:1) than the average of the other lines, 0.44:1. The whey resulting from the protein extraction of line 10 could find application in prebiotic products or for the production of alternative sweeteners. Uronic acid contents were high for lines 2, 10 and Harovinton (4.4%) and the lowest for line 7. A small amount of uronic acid was recovered in the whey fractions, suggesting that they were mainly soluble uronic acids.

Table 5 summarizes the composition of the fiber fraction, and the total soluble sugars are also indicated. Lines 11 and 12 had the highest total soluble sugar content (7.0%). In contrast, Harovinton and lines 2, 4, 5 presented the lowest total soluble sugar content, averaging 3.9% (see

Table 3
Monosaccharide composition of defatted flour from six selected varieties (% dry-weight basis)[A]

Variety	Rhamnose	Arabinose	Galactose	Glucose	Xylose	Mannose	Rham/Arab[B]	Rham/UA[C]
Harovinton	0.52 ± 0.01^{a}	2.35 ± 0.02^{b}	6.71 ± 0.03^{a}	5.25 ± 0.05^{a}	1.46 ± 0.02^{a}	$1.00 \pm 0.00^{b-d}$	0.222	0.065
Line 2	0.60 ± 0.01^{b}	2.56 ± 0.15^{c}	$7.74 \pm 0.36^{c,d}$	6.64 ± 0.57^{e}	1.48 ± 0.10^{a}	$0.95 \pm 0.03^{a-c}$	0.233	0.065
Line 4	0.51 ± 0.04^{a}	2.81 ± 0.04^{d}	6.97 ± 0.19^{a}	$5.67 \pm 0.11^{a-d}$	1.53 ± 0.13^{a}	0.81 ± 0.02^{a}	0.182	0.068
Line 6	0.62 ± 0.03^{b}	2.14 ± 0.06^{a}	$7.12 \pm 0.18^{a,b}$	$5.35 \pm 0.09^{a,b}$	1.40 ± 0.03^{a}	1.23 ± 0.14^{e}	0.290	0.079
Line 10	0.66 ± 0.04^{b}	2.55 ± 0.06^{c}	8.07 ± 0.49^{d}	$5.68 \pm 0.30^{b-d}$	1.36 ± 0.04^{a}	1.08 ± 0.06^{d}	0.259	0.076
Line 12	0.63 ± 0.07^{b}	2.50 ± 0.02^{c}	$7.53 \pm 0.08^{b,c}$	$5.49 \pm 0.02^{a-c}$	1.48 ± 0.48^{a}	$0.95 \pm 0.07^{a,b}$	0.251	0.083

Values are reported as mean value ± SD.
[A] Data are means of triplicate measurements.
[B] Ratio of rhamnose/arabinose.
[C] Ratio of rhamnose/uronic acids.
[a–e] Means in a column with different letters are significant different ($P < 0.05$).

Table 4
Composition of whey of 12 soybean lines (percentage dry-weight basis)[A]

Variety	Protein	Ash	Total CHO[B]	Sucrose	Raffinose	Stachyose	Total soluble sugars[C]	Uronic acids
Harovint	38.22 ± 0.09^{a}	$9.69 \pm 0.08^{c,d}$	52.09	12.15 ± 0.99^{g}	$1.89 \pm 0.04^{g,h}$	8.52 ± 0.01^{b}	$22.55 \pm 1.04^{e,f}$	4.85 ± 0.49^{e}
Line 2	37.70 ± 0.00^{a}	$9.56 \pm 0.04^{c,d}$	52.74	11.53 ± 0.39^{f}	$1.80 \pm 0.04^{f,g}$	9.96 ± 0.05^{g}	23.29 ± 0.39^{g}	$4.16 \pm 0.17^{d,e}$
Line 3	$39.52 \pm 0.01^{a,b}$	$9.76 \pm 0.18^{c,d}$	50.72	10.80 ± 0.18^{e}	2.00 ± 0.03^{h}	10.35 ± 0.04^{i}	$23.14 \pm 0.11^{f,g}$	$3.83 \pm 0.34^{b-d}$
Line 4	$40.89 \pm 0.13^{b,c}$	9.34 ± 0.15^{c}	49.77	9.19 ± 0.10^{c}	$1.30 \pm 0.05^{a-c}$	8.39 ± 0.01^{a}	18.89 ± 0.09^{b}	$3.25 \pm 0.34^{a,b}$
Line 5	$41.56 \pm 1.95^{b-d}$	$8.89 \pm 0.02^{b,c}$	49.55	11.57 ± 0.03^{f}	$1.86 \pm 0.04^{g,h}$	9.16 ± 0.01^{c}	$22.59 \pm 0.03^{e,f}$	$3.23 \pm 0.69^{a,b}$
Line 6	$41.87 \pm 0.83^{c,d}$	$9.55 \pm 0.10^{c,d}$	48.59	$10.39 \pm 0.29^{d,e}$	$1.81 \pm 0.28^{f,g}$	9.88 ± 0.09^{g}	22.08 ± 0.62^{e}	$3.19 \pm 0.93^{a,b}$
Line 7	$45.16 \pm 1.52^{e,f}$	$11.03 \pm 0.02^{d,e}$	43.81	5.52 ± 0.01^{a}	$1.40 \pm 0.05^{c,d}$	9.47 ± 0.04^{d}	16.39 ± 0.05^{a}	2.83 ± 0.69^{a}
Line 8	$41.55 \pm 2.46^{b-d}$	5.62 ± 1.92^{a}	52.83	9.21 ± 0.09^{c}	$1.29 \pm 0.08^{a-c}$	9.41 ± 0.04^{d}	19.92 ± 0.16^{c}	$3.86 \pm 0.31^{b-d}$
Line 9	$41.72 \pm 0.26^{b-d}$	12.30 ± 0.01^{e}	45.99	8.34 ± 0.01^{b}	$1.52 \pm 0.03^{d,e}$	$8.46 \pm 0.01^{a,b}$	18.33 ± 0.05^{b}	$3.40 \pm 0.27^{a-c}$
Line 10	$43.43 \pm 0.02^{d,e}$	9.24 ± 1.57^{c}	47.32	13.29 ± 0.02^{h}	$1.66 \pm 0.08^{e,f}$	10.24 ± 0.07^{h}	25.20 ± 0.10^{h}	$4.12 \pm 0.13^{c-e}$
Line 11	45.90 ± 0.06^{f}	7.71 ± 0.18^{b}	46.40	9.83 ± 0.05^{d}	$1.34 \pm 0.01^{b,c}$	9.70 ± 0.05^{f}	20.86 ± 0.08^{d}	$4.01 \pm 0.70^{c,d}$
Line 12	$42.01 \pm 0.15^{c,d}$	$9.75 \pm 0.31^{c,d}$	48.24	11.49 ± 0.04^{f}	1.16 ± 0.00^{a}	9.57 ± 0.07^{e}	22.22 ± 0.11^{e}	$3.92 \pm 0.56^{b-d}$

Values are reported as mean value ± SD.
[A] Data are means of triplicate measurements.
[B] Total carbohydrates determined by difference.
[C] Sum of sucrose, raffinose and stachyose.
[a–i] Means in a column with different letters are significant different ($P < 0.05$).

Table 5
Composition of fiber of 12 soybean lines (percentage dry-weight basis)[A]

Variety	Protein	Ash	Total CHO[B]	Sucrose	Raffinose	Stachyose	Total soluble sugars[C]	Uronic acids
Harovinton	$43.81 \pm 1.17^{b,c}$	$3.64 \pm 0.16^{c-e}$	52.55	1.83 ± 0.00^{c}	$0.51 \pm 0.00^{c,d}$	1.86 ± 0.03^{c}	4.20 ± 0.03^{c}	14.01 ± 0.36^{f}
Line 2	43.61 ± 0.83^{b}	$3.94 \pm 0.22^{e-g}$	52.45	1.46 ± 0.01^{b}	0.27 ± 0.00^{a}	1.51 ± 0.01^{a}	3.24 ± 0.02^{b}	13.27 ± 0.34^{f}
Line 3	55.18 ± 0.46^{h}	4.83 ± 0.26^{h}	39.99	2.98 ± 0.02^{h}	0.73 ± 0.00^{f}	2.86 ± 0.03^{f}	6.58 ± 0.05^{g}	8.62 ± 0.24^{a}
Line 4	43.00 ± 1.03^{b}	2.80 ± 0.34^{a}	54.20	1.16 ± 0.04^{a}	0.26 ± 0.01^{a}	1.62 ± 0.02^{b}	3.04 ± 0.04^{a}	13.83 ± 0.64^{f}
Line 5	45.52 ± 0.28^{d}	3.22 ± 0.09^{b}	51.26	2.29 ± 0.03^{d}	0.63 ± 0.01^{e}	2.06 ± 0.02^{d}	4.98 ± 0.06^{d}	11.68 ± 0.77^{e}
Line 6	46.96 ± 0.45^{f}	$4.19 \pm 0.11^{f,g}$	48.86	2.82 ± 0.02^{g}	0.66 ± 0.02^{e}	2.61 ± 0.03^{e}	6.09 ± 0.07^{f}	$10.68 \pm 0.51^{c,d}$
Line 7	$45.71 \pm 0.86^{d,e}$	4.29 ± 0.17^{g}	50.00	2.50 ± 0.08^{e}	0.47 ± 0.02^{c}	2.91 ± 0.11^{f}	5.88 ± 0.21^{e}	$9.73 \pm 0.91^{b,c}$
Line 8	$46.59 \pm 0.67^{e,f}$	$3.39 \pm 0.12^{b-d}$	50.02	2.58 ± 0.05^{f}	0.41 ± 0.06^{b}	2.90 ± 0.00^{f}	5.89 ± 0.01^{e}	$11.44 \pm 0.38^{d,e}$
Line 9	43.59 ± 0.29^{b}	$3.73 \pm 0.04^{d,e}$	52.68	3.32 ± 0.02^{k}	$0.50 \pm 0.03^{c,d}$	2.98 ± 0.01^{g}	6.79 ± 0.03^{h}	$10.81 \pm 0.36^{d,e}$
Line 10	41.09 ± 0.73^{a}	$3.10 \pm 0.25^{a,b}$	55.81	3.07 ± 0.02^{i}	0.53 ± 0.00^{d}	$2.92 \pm 0.03^{f,g}$	6.52 ± 0.05^{g}	8.18 ± 0.66^{a}
Line 11	49.10 ± 0.60^{g}	$3.81 \pm 0.06^{e,f}$	47.09	$3.02 \pm 0.01^{h,i}$	0.52 ± 0.03^{d}	3.46 ± 0.02^{i}	7.00 ± 0.03^{i}	$8.83 \pm 0.62^{a,b}$
Line 12	44.55 ± 0.86^{c}	$3.33 \pm 0.15^{b,c}$	52.12	3.21 ± 0.02^{j}	0.53 ± 0.00^{d}	3.27 ± 0.03^{h}	7.02 ± 0.04^{i}	$11.40 \pm 0.28^{d,e}$

Values are reported as mean value ± SD.
[A] Data are means of triplicate measurements.
[B] Total carbohydrates determined by difference.
[C] Sum of sucrose, raffinose and stachyose.
[a–k] Means in a column with different letters are significant different ($P < 0.05$).

also Fig. 3). Lines 9 and 12 had the highest amount of sucrose and line 4 the lowest. Lines 2 and 4 showed the lowest (1.8%) and lines 11 and 12 had the highest (3.9%) amount of raffinose and stachyose in the fiber. The presence of soluble sugars in the fiber fraction indicates that a minor content of soluble sugars were not removed during the filtering step of SPI making. Most of the uronic acids were recovered in the fiber fraction as shown in Fig. 4. The uronic acids ranged from 8.6% to 14% in the fiber fraction. Harovinton and lines 2 and 4 had the highest uronic acid concentration (14%) and lines 3 and 10 had the lowest (8.4%). The total uronic acids as a percentage of total carbohydrates showed differences as well. For instance, line 10 had only 15% in contrast to 27% uronic acid for Harovinton. Lines 7, 9 and 11 averaged 19.6% and the other lines averaged 23%.

Table 6 summarizes the monosaccharide composition of the fiber fraction of six lines (the soluble sugars were removed). When comparing the defatted flours and the fibers, it is observed that most of the rhamnose, arabinose, xylose and uronic acids were recovered in the fiber by-product. This indicated the presence of high amount of insoluble carbohydrate (hemicellulose) and pectins. Line 12 showed the highest content of xylose (6.0%) and line 2 and Harovinton showed the lowest (3.0%). Line 10 presented the highest galactose content in both the defatted flour and fiber. Lines 10 and 12 had the highest galactose, xylose and glucose contents compared to the other four lines analyzed. The ratio of rhamnose to arabinose was significantly different, ranging from 0.187:1 for lines 2, 4 and Harovinton, to 0.344:1 for lines 6, 10 and 12. In addition, lines 2, 4 and Harovinton had 0.065:1 rhamnose to uronic acid ratio,

Table 6
Monosaccharide composition of washed fiber from 6 selected varieties (% dry-weight basis)[a]

Variety	Rhamnose	Arabinose	Galactose	Glucose	Xylose	Mannose	Rham/Arab[b]	Rham/UA[c]
Harovinton	0.87 ± 0.04^{a}	4.70 ± 0.10^{b}	7.29 ± 0.14^{c}	1.68 ± 0.32^{a}	3.07 ± 0.08^{a}	$1.31 \pm 0.10^{a,b}$	0.185	0.621
Line 2	0.88 ± 0.04^{a}	4.69 ± 0.10^{b}	$7.21 \pm 0.14^{b,c}$	2.26 ± 0.14^{b}	3.01 ± 0.15^{a}	1.24 ± 0.02^{a}	0.187	0.660
Line 4	0.92 ± 0.04^{a}	$4.89 \pm 0.14^{b,c}$	6.08 ± 0.16^{a}	1.69 ± 0.10^{a}	3.78 ± 0.11^{b}	1.49 ± 0.08^{b}	0.189	0.668
Line 6	1.23 ± 0.06^{b}	3.59 ± 0.13^{a}	6.90 ± 0.18^{b}	$2.65 \pm 0.13^{b,c}$	4.84 ± 0.23^{c}	2.78 ± 0.06^{c}	0.344	0.116
Line 10	1.63 ± 0.04^{c}	4.98 ± 0.10^{c}	9.86 ± 0.21^{e}	3.50 ± 0.09^{d}	5.46 ± 0.36^{d}	2.77 ± 0.35^{c}	0.328	0.200
Line 12	1.72 ± 0.03^{d}	$4.80 \pm 0.30^{b,c}$	8.22 ± 0.49^{d}	2.91 ± 0.62^{c}	6.01 ± 0.30^{e}	2.68 ± 0.06^{c}	0.359	0.151

Values are reported as mean value ± SD.
[a] Data are means of triplicate measurements.
[b] Ratio of rhamnose/arabinose.
[c] Ratio of rhamnose/uronic acids.
[a–e] Means in a column with different letters are significant different ($P < 0.05$).

while the other lines averaged 0.155:1. These results are in agreement with the data obtained from the defatted flour samples, where the ratio of rhamnose/uronic acids was higher for lines 6, 10, 12 than for the other lines analyzed. These results are also in agreement with the high content of uronic acids found in the fiber fractions (Table 5 and Fig. 4), suggesting that the rhamnose and arabinose residues may derive from the acid-hydrolyzed pectin fractions. Galactose, arabinose and uronic acids were the major monosaccharides present in the acid-hydrolyzed fiber. Glucose was present in low amounts, averaging 2.9%. These results are in agreement with those previously reported by Schols and Voragen (1998) who showed that the pectin-rich fraction from soybean was mainly constituted of galactose, arabinose and uronic acids. Xylose was also recovered in the fiber fractions (3–6%), probably derived from the acid hydrolysis of hemicellulose, xylans (β-1-4-linked polymers of xylose) and xyloglucans (β-1-4-linked polymers of glucose with xylose side chains), carbohydrates with limited water solubility. The fiber fractions also showed low amounts of glucose resulting from the acid-hydrolyzed xyloglucans, as most glucose was recovered in the whey by-product as shown in Fig. 3 and Table 4.

4. Conclusions

Although these lines were bred to have altered protein profiles, these results show significant differences in the amounts and percentage ratios of carbohydrates among the parent variety Harovinton and its 11 derived lines.

Defatted flours, fibers and wheys showed significant differences in the soluble sugars, monosaccharide composition and uronic acids contents. The relative amounts of sucrose, raffinose and stachyose varied among the lines. The fiber and whey, both by-products of SPI production, were rich in uronic acids and soluble sugars, respectively. The main constituent sugars of defatted flours and fibers were galactose, glucose, arabinose and uronic acids. The results indicate that some lines are richer in pectins than other lines. Furthermore, the rhamnose/arabinose and rhamnose/uronic acid percentage ratios suggest that some lines may have more branched uronic acids than others.

Tailoring breeding of soybean lines for particular applications in the soy-food industry is becoming increasingly important, especially for the optimization of soybean raw material applications. The results of this study are a clear example of the great potential of varietal differences. For instance, defatted flour and whey of lines 10 and 12 may find uses when developing specific soy foods when high sucrose content is a desirable trait. Fiber by-products may be incorporated as a source of soluble and insoluble carbohydrates in food products. The whey by-product of these lines, in particular line 10, may find uses as a source of raffinose and stachyose for prebiotic products and for production of soy oligosaccharide syrups.

Acknowledgments

The authors thank Yolanda Brummer for the technical training and assistance during experiments. This work was supported by funding provided by Ontario Soybean Growers and Agriculture and Agri-Food Canada (Matching Investment Initiatives).

References

Aspinall, G. O. (1988). Chemistry of soyben carbohydrates. In *Soybean utilization alternatives: A symposium sponsored by The Center for Alternative Crops and Products* (pp. 117–129). St. Paul, MN: The Center for Alternate Crops and Products.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*(2), 484–489.

Carrol, K. K. (1991). Review of clinical studies on cholesterol-lowering response to soy protein. *The Journal of American Dietetic Association, 91*(7), 820–825.

Cristofaro, E., Mottu, F., & Wuhrmann, J. J. (1974). Involvement of the raffinose family of oligosaccharides in flatulence. In H. L. Sepple & K. W. McNutt (Eds.), *Sugars in nutrition* (pp. 313–363). New York: Academic Press.

Giannoccaro, E., Wang, Y., & Chen, P. (2006). Effects of solvent, temperature, time, solvent-to-sample ratio, sample size, and defatting on the extraction of soluble sugars in soybean. *Journal of Food Science, 71*(1), C59–C64.

Hymowitz, T., Walker, W. M., Collins, F. I., & Panczner, J. (1972a). Stability of sugar content in soybean strains. *Communications in Soil Science and Plant Analysis, 3*, 367–373.

Hymowitz, T., Walker, W. M., Collins, F. I., & Panczner, J. (1972b). Relationship between the content of oil, protein, and sugar in soybean seed. *Agronomy Journal, 64*, 613–615.

Liu, K. (1999). Chemistry and nutritional value of soybean components. *Soybeans: Chemistry, technology and utilization* (pp. 72–76). Gaithersburg, MD: Aspen Publisher Inc..

Mullin, W. J., & Xu, W. (2000). A study of the intervarietal differences of cotyledon and seed coat carbohydrates in soybean. *Food Research International, 33*, 883–891.

Poysa, V., Woodrow, L., & Yu, K. (2006). Effect of soy protein subunit composition on tofu quality. *Food Research International, 39*(3), 309–317.

Poysa, V., & Woodrow, L. (2002). Stability of soybean seed composition and its effect on soymilk and tofu yield and quality. *Food Research International, 35*(4), 337–345.

Rackis, J. J. (1975). Oligosaccharides of food legumes: Alpha-galactosidase activity and flatus problem. In A. Jeanes & J. Hodge (Eds.), *Physiological effects of food carbohydrates* (pp. 207–222). Washington, DC: American Chemical Society.

Renkema, J. M. S., Lakemond, C. M. M., de Jongh, H. H. J., Gruppen, H., & van Vliet, T. (2000). The effect of pH on heat denaturation and gel forming properties of soy proteins. *Journal of Biotechnology, 79*(3), 223–230.

Roberfroid, M. (2007). Prebiotics: the concept revisited. Effects of probiotics and prebiotics. *The Journal of Nutrition*(137), 830S–837S.

Schols, H. A., & Voragen, A. G. J. (1998). Cell wall polysaccharides from soybean (Glycine max.) meal. Isolation and characterisation. *Carbohydrate Polymers, 37*(1), 87–95.

Slavin, J. (1988). What is dietary fiber? In *Soybean utilization alternatives: A symposium sponsored by The Center for Alternative Crops and Products* (pp. 179–188). St. Paul, MN: The Center for Alternate Crops and Products.

Taira, H. (1990). Quality of soybean for processed foods in Japan. *Japan Agricultural Research Quarterly*(24), 224–230.

Tomomatsu, H. (1994). Health effects of oligosaccharides. *Food Technology*(48), 61–65.

Tsai, A. C., Mott, E. L., Owen, G. M., Bennick, M. R., Lo, G. S., & Steinke, F. H. (1983). Effects of soy polysaccharide on gastrointestinal functions, nutrient balance, steroid excretions, glucose tolerance, serum lipids, and other parameters in humans. *The American Journal of Clinical Nutrition, 38*, 504–511.

Zarkadas, C. G., Gagnon, C., Poysa, V., Khanizadeh, S., Cober, E. R., Chang, V., et al. (2007). Protein quality and identification of the storage protein subunits of tofu and null soybean genotypes, using amino acid analysis, one- and two-dimensional gel electrophoresis, and tandem mass spectrometry. *Food Research International, 40*(1), 111–128.