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ORIGINAL PAPER

Effect of genotype and environment on the surface characteristics and functionality of air-classified faba bean protein concentrates

M. Martinez[1] · A. K. Stone[1] · A. G. Yovchev[1] · R. Peter[1] · A. Vandenberg[2] · M. T. Nickerson[1]

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Abstract The overall goal of this research was to examine the effect of genotype and environment on the ratio of legumin to vicilin proteins (*L*/*V*), the surface characteristics and the functionality of air-classified faba bean protein concentrates. Seeds of five faba bean (*Vicia faba*) genotypes (CDC-Fatima, Taboar, SSNS-1, FB9-4 and FB18-20) grown in 2012 at two field plot locations, Melita and Morden (Manitoba, Canada), were used. *L*/*V* ratios ranged from 3.76 to 5.40 depending on the genotype and the environment, as only the effect of environment was found to be significant ($p < 0.05$). The *L*/*V* ratio for concentrates produced from seeds grown in Melita was greater than those grown in Morden. The effect of genotype and the interaction effect between genotype and environment on the surface charge were also found to be significant ($p < 0.05$). The protein concentrates' surface charge ranged between −19.4 and −31.8 mV (at pH 7.0). The effect of environment on the interfacial tension was significant ($p < 0.001$), as the values for protein concentrates were found to be lower for those produced from seeds grown in Melita (7.9 mN/m) than those grown in Morden (10.2 mN/m). No significant effects were found for the surface tension and foaming capacity in response to either genotype or environment. The foam stability was influenced by the environment and by the interaction between genotype and environment ($p < 0.05$). The effects of genotype and environment on the emulsion stability, oil-holding capacity and water hydration capacity were insignificant. As for the protein concentrates' solubility, the effect of genotype and the interaction between genotype and environment were significant, with values of solubility ranging between 82.3 and 88.0 %.

Keywords Faba bean · Genotype · Environment · Air classification · Functionality

Introduction

Within the protein ingredient market, trends are shifting away from animal-derived proteins toward other plant-based alternatives other than soy or wheat, due to their low cost, nutrition, consumer-perceived fears (e.g., prions), low allergens and changing dietary preferences based on moral and religious beliefs. Faba bean protein products have the potential to fill some of the market demands based on their nutritious attributes and functionality (as an ingredient), making it an attractive alternative to soy in vegan products. Faba beans contain high amounts of carbohydrate (~55 %), protein (~25–30 %), minerals (calcium, magnesium and iron) and vitamins (thiamine, riboflavin and pyridoxine) and are low in fat (<1 %) [1].

Similar to most of the pulses, the majority of the proteins found in faba beans can be divided into globulin and albumin fractions, where globulins account for the main storage proteins (60 %) and the albumins account (20 %) for most of the regulatory proteins (metabolic activities and enzymatic regulations) [2]. The globulin proteins are further subdivided into both an 11S legumin (molecular mass of ~350–400 kDa) and 7S vicilin (molecular mass of ~150 kDa) fraction, similar to what is found in other pulse crops. Faba bean 11S proteins have large proportions of

✉ M. T. Nickerson
michael.nickerson@usask.ca

[1] Department of Food and Bioproduct Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada

[2] Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada

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arginine, glutamic acid and aspartic acid, but are low in methionine and cysteine [3]. Faba bean vicilins (7S) contain a variety of polypeptide fragments (15–66 kDa) and are also glycosylated [3]. Faba beans also contain glycosides such as vicine and convicine, which are responsible for a number of anti-nutritive effects in both animals and humans. In animals, they result in a decrease in egg weight, hatchability and quality [4–7], whereas in humans they are known for causing favism (a type of anemia) [8, 9]. However, their content can be reduced to a negligible amount via processing or agronomic practices.

Air classification is a process that physically separates (and enriches) fractions, such as proteins and starches based on differences in specific gravities between particles. Protein concentrates are typically classified as having protein levels <65 %; however, they are usually produced at levels closer to 50 % depending on the seed, environmental factors and processing conditions. The quality of the concentrate is highly dependent on the protein chemistry (amino acid composition, level of folding, surface characteristics), processing conditions and level of denaturation endured, as well as the presence of non-proteinaceous ingredients such as lipids, phenolic compounds and starches.

The overall goal of this study is to examine the effect of faba bean genotype and the environment on the ratio of legumin to vicilin proteins, the surface characteristics and the functionality of air-classified protein concentrates. The functional attributes of the faba bean protein concentrates originating from breeder seeds will also be contrasted with a select number of commercial concentrate ingredients. Findings from this study could indicate whether greater consideration should be paid to the raw material entering secondary processing facilities to produce the faba bean protein concentrate ingredient for market.

Materials and methods

Materials

Seeds of five faba bean (*Vicia faba*) genotypes (CDC-Fatima, Taboar, SSNS-1, FB9-4 and FB18-20) grown in 2012 at two field plot locations, Melita and Morden (MB, Canada), were kindly donated by the Crop Development Centre (University of Saskatchewan, Saskatoon, SK, Canada). SSNS-1 and Taboar have small seeds; Fatima is an intermediate size seed; FB9-4 and FB18-20 are considered to be large seeds. The following commercial protein concentrates were used in this study: soy (Arcon S; Archer Daniels Midland Co., Chicago, IL, USA), faba bean (Parrheim Foods, Saskatoon, SK, Canada), great northern bean (Parrheim Foods, Saskatoon, SK, Canada) and pea (Progress Protein; Parrheim Foods, Saskatoon, SK, Canada).

All chemicals used in this study were of reagent grade except for sodium dodecyl sulfate, Tris and glycine, which were of electrophoresis purity and purchased from Sigma-Aldrich Co. (Oakville, ON, Canada). The deionized water used in this research was from a Millipore Milli-Q™ water purification system (Millipore Corp., Milford, MA, USA).

Preparation of faba bean flours and protein concentrates

Prior to air classification to produce the protein concentrates, seeds were de-hulled using a Satake mill (Satake, Penrith, NSW, Australia), ground into coarse flour using a disk mill (Glen Mills Inc., Clifton, NJ, USA) and then milled using a Hosokawa Alpine 100 UPZ pin mill at 20,000 rpm. One hundred grams of the pin-milled flour was then air-classified using a 100 MZR Hosokawa Alpine Air Classifier (Augsburg, Germany) at 12,000 rpm and flow rate of 64 m^3/h to obtain protein and starch fractions. Protein levels were determined by Official Method 990.03 of AOAC International [10] using a FP-528PC Protein/Nitrogen Analyzer (LECO Corp., St. Joseph, MI, USA) and a nitrogen conversion factor of 6.25.

Legumin/vicilin ratio determination

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions was performed for all flour and concentrate samples to identify the relative proportion of legumin and vicilin [(reported as the legumin/vicilin (*L*/*V*) ratio)]. Separation of legumin and vicilin fractions was carried out via SDS-PAGE according to Laemmli [11] with some modifications. In brief, 75 μL of a 3 mg/mL flour–water (or 1 mg/mL concentrate water) suspension was diluted with 75 μL of water to give a final sample concentration of 0.5 % (v/v). To all samples, 142.5 μL of Laemmli sample buffer (65.8 mM Tris–HCl, 2.1 % SDS, 26.3 % (w/v) glycerol, 0.01 % bromophenol blue, pH 6.8) (BIO-RAD, Mississauga, ON, Canada) and 7.5 μL of β-mercaptoethanol were added. The samples then were vortexed for 10 s. All samples were heated at 95 °C for 5 min using an Incu Block model 285 (Denville Scientific Inc., South Plainfield, NJ, USA) and then centrifuged using an Eppendorf Centrifuge 5424 (Hamburg, Germany) at 12,000×*g* for 5 min. Precast 4–20 % Precise Tris–glycine gels (Thermo Scientific, Rockford, IL, USA) were used to run the samples in a MGV-202 Vertical Mini-Gel System (CBS Scientific, San Diego, CA, USA) for a period of ~40 min at 200 V using a Power Source 300 V Electrophoresis Power Supply (VWR, Mississauga, ON, Canada). Precision Plus Protein™ Prestained Standards (BIO-RAD) ranging from 10 to 250 kDa were used as molecular markers. Gels were then stained using Phastgel Blue R tablets (GE Healthcare Life Sciences, Uppsala,

Sweden) for 1 h, followed by destaining with methanol/water/acetic acid (3:6:1) three times over a 24-h period. The destained gels were then scanned using an EPSON Perfection V750 Pro scanner (EPSON, Markham, ON, Canada). Quantification of bands from the SDS-PAGE analysis was carried out by implying densitometry using Image J software (National Institutes of Health, Bethesda, MD, USA). All samples were run in triplicate. The *L*/*V* ratio was reported as mean ± one standard deviation.

Amino acid profile

The amino acid composition of the protein concentrates from one field replicate, from seeds grown only in Morden, MB, were used to examine differences among faba bean cultivars. All analyses were performed at POS Bio-Sciences using a Pico-Tag amino acid analysis system (Waters Corporation, Milford, MA, USA) and high-performance liquid chromatography (HPLC). In general, 15 amino acid residues were quantified according to the method developed by Bidlingmeyer et al. [12], which involves adding 15 mL of 6N HCl to ~20 mg of samples and holding at 110 °C for 20 h to hydrolyze the proteins into individual amino acids prior to HPLC separation. The amount of sulfur-containing amino acids was determined according to Official Method 985.28 of AOAC International [10] with some modifications, in which the addition of 1-octanol was omitted; 10 mL of cold performic acid was added to oxidize cysteine and methionine overnight at 4 °C, prior to protein hydrolysis with 15 mL of 6 N HCl at 110 °C for 16 h. The quantity of tryptophan was determined according to Official Method 988.15 of AOAC International [10] with modifications, in which samples were hydrolyzed by treating with 10 M NaOH in a boiling water bath for 20 min and then in an oven at 110 °C for 16 h prior to HPLC separation. All analyses were performed in duplicate.

Surface characteristics of the protein concentrates

Prior to the testing, protein concentrate solutions of various concentrations were prepared by dispersing the isolates (weight corrected on the basis of protein content) in 10 mM sodium phosphate buffer (pH 7.0) (w/w). The pH of the resulting solution was then adjusted to 7.0 with either 0.1 M NaOH or 0.1 M HCl followed by overnight stirring (500 rpm, 4 °C, ~16 h).

Surface charge (zeta potential)

Zeta potential was measured for 0.05 % (w/w) protein solutions using a Zetasizer Nano-ZS90 (Malvern Instruments, Westborough, MA, USA). The electrophoretic mobility (U_E) of protein solutions gives the zeta potential (ζ, mV) when using Henry's equation:

$$U_E = \frac{2\varepsilon \times \zeta \times f(k\alpha)}{3} \quad (1)$$

Here, ε is permittivity (F (Farad)/m), $f(k\alpha)$ is a function associated with the ratio of particle radius (α) to the Debye length (k), and η is the viscosity (mPa s) of the solution. The Smoluchowski approximation $f(k\alpha)$ for this study was set to 1.5. All measurements are reported as mean ± one standard deviation ($n = 2$).

Surface hydrophobicity

Surface hydrophobicity was determined according to a modification of the method of Kato and Nakai [13] using the fluorescent probe 8-anilino-1-naphthalenesulfonic acid (ANS). Protein isolates were dispersed (0.025 % w/w) in 10 mM sodium phosphate buffer (pH 7.0) by stirring overnight at 4 °C, after which dilutions of 0.005, 0.010, 0.015 and 0.020 % protein were made in 10 mM sodium phosphate buffer (pH 7.0). For each protein concentration (0.005–0.025 %, 1.6-mL samples), 20 μL of 8 mM ANS solution (in 10 mM sodium phosphate buffer, pH 7.0) was added; samples then were vortexed for 10 s and kept in the dark for 5 min. Fluorescence intensity was measured using a Fluoro-Max-4 spectrophotometer (Horiba Jobin–Yvon Inc., Edison, NJ, USA) with excitation and emission wavelengths of 390 and 470 nm, respectively, and slit widths of 1 nm. Fluorescence intensity values for the ANS blank and protein blanks were subtracted from the fluorescence intensity of the protein solutions containing ANS. The initial slope (S_0) of the plot of the fluorescence intensity against protein concentration was calculated by linear regression analysis and used as an index of the protein surface hydrophobicity (S_0-ANS). All measurements are reported as mean ± one standard deviation ($n = 2$).

Interfacial and surface tension

Interfacial tension between 0.25 % (w/w) protein concentrate solutions and canola oil and between protein solutions and the air interface were determined using the du Noüy ring and a semiautomatic tensiometer (Lauda TD2, GmbH & Co., Lauda-Königshofen, Germany). Interfacial tension calculated between Milli-Q water (without protein) and canola oil, and between deionized water (without protein) and the air interface served as the controls. Interfacial tension (γ) then was calculated from the equation below using the maximum force (F_{max}).

$$\gamma = \frac{F_{max}}{4\pi R\beta} \quad (2)$$

Here, γ is the interfacial tension (mN/m), R is the radius of the ring (20 mm), and β is a correction factor which is dependent on the dimensions of the ring and the density of

the liquid. All measurements are reported as mean ± one standard deviation ($n = 2$).

Functional attributes of the flours and concentrates

The sample preparation for all functional tests was similar to that described in the surface properties section.

Foaming

Foaming capacity (FC) and foam stability (FS) for 1.0 % (w/w) protein flour/concentrate solutions were determined according to Liu et al. [14]. In brief, 15 mL of each protein solution was transferred to a 400-mL beaker for homogenization using an Omni Macro Homogenizer (Omni International, Marietta, GA, USA) with a 20-mm sawtooth probe at speed 4 (~7200 rpm) for 5 min. The homogenized sample was then transferred immediately to a 100-mL graduated cylinder and the foam volume measured at time 0 and at 30 min, with FC and FS determined using Eqs. 3 and 4, respectively:

$$\mathrm{FC} = \frac{V_{\mathrm{F0}}}{V_{\mathrm{sample}}} \times 100\,\% \tag{3}$$

$$\mathrm{FS} = \frac{V_{\mathrm{F30}}}{V_{\mathrm{F0}}} \times 100\,\% \tag{4}$$

where V_{F0} is the volume of the foam at time 0 min, V_{sample} is the initial volume of sample used (15 mL), and V_{F30} is the foam volume after 30 min. All measurements are reported as mean ± one standard deviation ($n = 2$).

Emulsion stability

Emulsion stability (ES) for 1.0 % (w/w) protein flour/concentrate solutions was measured by homogenizing 5 mL of protein flour/concentrate solution with 5 mL of canola oil using an Omni Macro Homogenizer equipped with a 20-mm sawtooth probe at speed 4 (~7200 rpm) for 5 min. The emulsion formed was immediately transferred to a 10-mL graduated cylinder and observed for separation of the aqueous phase from the turbid phase of the emulsion after 30 min. The emulsion stability was calculated using Eq. 5:

$$\mathrm{ES} = \frac{V_{\mathrm{B}} - V_{\mathrm{A}}}{V_{\mathrm{A}}} \times 100\,\% \tag{5}$$

where V_{B} is the volume of the aqueous phase before homogenization (5 mL) and V_{A} is the volume of the aqueous phase after homogenization.

Oil-holding capacity and water hydration capacity

Oil-holding capacity (OHC) was determined according to Chakraborty [15] with slight modifications. In brief, 10 g of canola oil was combined with 1 g of protein flour/concentrate (adjusted based on protein content) in a weighed centrifuge tube. The mixture was vortexed for 10 s every 5 min for a total duration of 30 min, followed by centrifuging at 1000×*g* (VWR Clinical 200, VWR International, Mississauga, ON, Canada) for 15 min and then decanting of the supernatant. The OHC was calculated according to Eq. 6:

$$\mathrm{OHC} = \frac{Wt_{\mathrm{Wet}} - Wt_{\mathrm{Dry}}}{Wt_{\mathrm{Dry}}} \times 100\,\% \tag{6}$$

where Wt_{Wet} and Wt_{Dry} are the weights of the wet and dry samples, respectively. All measurements are presented as mean ± one standard deviation ($n = 2$). Water hydration capacity (WHC) was determined in a similar manner, except that the canola oil was substituted with water.

Solubility

Protein solubility for a 1.0 % (w/w) solution was determined according to Can Karaca et al. [16] with slight modifications. In brief, 20 g of protein concentrate solution was transferred to a 50-mL centrifuge tube followed by centrifugation (9100×*g*, 10 min, 4 °C) using a Sorvall RC-6 Plus Superspeed Centrifuge (Thermo Scientific, Asheville, NC, USA). Then, 15 g of the supernatant obtained was taken for determination of the protein content using a K-355 digestion and nitrogen distillation unit (Buchi, Flawil, Switzerland). The protein contents of the original samples were calculated on the basis of 0.2 g protein concentrate samples. A nitrogen-to-protein conversion factor of 6.25 was employed. Percent protein solubility was calculated by dividing the protein content of the supernatant by the protein content of the original sample and multiplying by 100 %.

Statistics

A two-way analysis of variance (ANOVA) was performed to test the main effects of genotype and the environment, along with their associated interaction on the *L*/*V* ratio, and the physicochemical and functional properties of the faba bean protein concentrates. SPSS software (IBM Corporation, Armonk, NY, USA) was used for all statistical analysis.

Results and discussion

Air classification of faba bean

Air classification is a milling process that relies on the physical separation of flours or streams due to differences in specific gravities between particles. Grains or seeds are

milled first into a very fine flour and then classified by means of airflow into light fine fraction (protein enriched) and heavy coarse fraction (starch enriched) [17]. Pinned milled faba bean flours of different genotypes grown in different locations were air-classified to yield both coarse (rich in starch) and fine (rich in protein) fractions (Table 1). Although there was a slight variability in protein levels and processing yields of the flours and the fractions between the genotypes, they were not considered to be significant ($p > 0.05$). Protein levels (on a dry weight basis) in the pinned milled flours ranged between 30.8 and 31.8 %. Upon air classification, the coarse and fine fractions had average yields of 68.3 and 26.2 %, respectively, with corresponding protein levels of 16.2 and 65.6 % (d.b.). For a 100 g initial flour sample entering the air classifier (avg. 31.1 g protein), 11.0 and 17.1 g of the protein was recovered in the coarse and fine fractions, respectively, to give process recovery efficiency for protein of 90.5 %.

Protein composition within faba bean protein concentrates

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-Page) under reducing conditions was used to investigate the protein profiles within the protein-classified materials. Using densitometry, the relative percentages and composition of each can be determined. A representative SDS-PAGE gel and corresponding densitometry analysis for protein concentrate material produced from *var*. SSNS-1, grown in Melita, are shown in Fig. 1. The *L*/*V* ratio was determined as the relative percentage of (L) [α-legumin chain (38 kDa) + β-legumin chain (24 kDa)] and vicilin (V) (54 kDa) proteins. Band identification revealed that the major bands were located at molecular masses of ~73, ~54, ~38 and ~24 kDa and were presumed to correspond to convicilin, vicilin, α-legumin and β-legumin chains, respectively. *L*/*V* ratios ranged from 3.76 to 5.40 depending on the genotype and the environment (Table 2). Only the effect of environment on *L*/*V* ratio was found to be significant ($p < 0.05$) (Table 3). Overall, the *L*/*V* ratio for concentrates produced from seeds grown in Melita (*L*/V ratio = 4.72) was greater than those from seeds grown in Morden (*L*/*V* ratio = 4.14).

The amino acid profiles as a function of genotype for concentrates produced from seeds grown only in Morden, MB, were relatively similar, despite some minor differences (Table 4). The average percentage of amino acids (proteins) found per gram of concentrate was 59.6 % (ranged between 54.9 and 66.2 %). Furthermore, the relative amount of neutral, charged and hydrophobic amino acids was 11.3 % (ranged between 10.7 and 12.2 %), 30.0 % (ranged between 27.9 and 33.3 %) and 18.3 % (ranged between 16.2 and 20.7 %), respectively (Table 4).

Table 1 Yields, protein levels and percent recovery for the starch and protein rich fractions of air-classified faba beans of varying genotypes

Genotype	Coarse fraction yield (%)	Fine fraction yield (%)	Protein content in the flour (%, db)	Protein content in the coarse fraction (%, db)	Protein content in the fine fraction (%, db)	Recovered protein (g) within the coarse fraction	Recovered protein (g) within the fine fraction	Total protein recovery (%)
SSNS-1	68.0 ± 4.6	27.0 ± 2.4	31.8 ± 1.4	16.4 ± 1.2	66.4 ± 1.3	11.1 ± 1.5	18.0 ± 1.6	91.7 ± 4.3
Taboar	68.7 ± 3.2	25.2 ± 2.4	31.5 ± 0.8	16.0 ± 1.3	66.6 ± 0.4	11.0 ± 1.4	16.7 ± 1.6	89.1 ± 5.2
Fatima	68.7 ± 3.2	25.2 ± 2.4	31.5 ± 0.8	16.0 ± 1.3	66.6 ± 0.5	11.0 ± 1.4	16.7 ± 1.6	89.1 ± 5.2
FB 9-4	66.7 ± 6.0	27.7 ± 2.5	31.7 ± 1.1	16.6 ± 2.7	64.2 ± 2.7	11.0 ± 2.9	17.6 ± 1.3	91.4 ± 5.3
FB 18-20	69.4 ± 4.2	26.1 ± 2.5	30.8 ± 1.5	15.9 ± 2.4	64.0 ± 1.5	11.0 ± 2.1	16.6 ± 1.3	91.1 ± 7.7
Average	68.3	26.2	31.4	16.2	65.6	11.0	17.1	90.5

Data represent mean values ± one standard deviation from seeds harvested from both locations, air-classified in two separate runs ($n = 4$). No significant differences were observed between growing locations, air classification runs or genotypes for any of the processing parameters ($p > 0.05$)

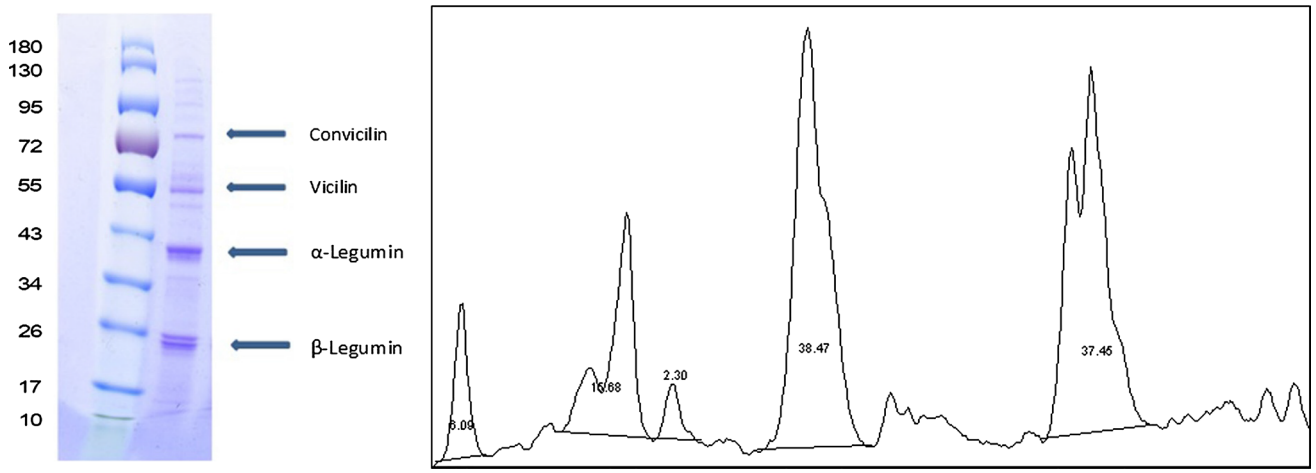


Fig. 1 Representative example of a SDS-PAGE gel (*left*) and corresponding densitometry analysis for faba bean protein concentrate prepared from *var.* SSNS-1 grown in Melita, MB

Table 2 Legumin/vicilin ratios for faba bean protein concentrates as a function of genotype and environment (Morden vs. Melita, MB)

Genotype	Legumin/vicilin ratio	
	Morden	Melita
SSNS-1	4.19 ± 0.08	4.55 ± 0.04
Taboar	3.76 ± 0.21	4.17 ± 0.29
Fatima	3.96 ± 0.27	4.62 ± 0.57
FB 9-4	4.21 ± 0.93	5.40 ± 0.54
FB 18-20	4.60 ± 0.40	4.88 ± 0.18

Data represent mean values ± one standard deviation from duplicate processing runs ($n = 2$)

Table 3 Effects of genotype and environment on physicochemical and functional properties of air-classified faba bean protein concentrates

Genotype (*G*)	Environment (*E*)		G × E
a) Physicochemical properties			
Legumin/vicilin ratio	NS	*	NS
Zeta potential	*	NS	*
Surface hydrophobicity	NS	NS	NS
Interfacial tension	NS	**	NS
Surface tension	NS	NS	NS
b) Functional properties			
Foaming capacity	NS	NS	NS
Foam stability	NS	*	*
Emulsion stability	NS	NS	NS
Oil-holding capacity	NS	NS	NS
Water hydration capacity	NS	NS	NS
Solubility	**	NS	*

NS not significant

* $p < 0.05$; ** $p < 0.001$

Similar to other pulses, faba bean samples were high in lysine (avg. 4.0 %) and low in cysteine (avg. 0.7 %) and methionine (avg. 0.4 %) (Table 4).

Surface characteristics of faba bean protein concentrates as a function of genotype and environment

The surface charge (zeta potential), hydrophobicity, interfacial tension and surface tension for various faba bean protein concentrates as a function of genotype and environment are given in Table 5. The protein concentrates' surface charge ranged between −19.4 to −31.8 mV (at pH 7.0). The effect of genotype and the interaction effect between genotype and environment on the surface charge were found to be significant ($p < 0.05$), whereas the main effect of environment was not (Table 3). For concentrates produced from seeds grown in Morden, MB, the surface charge was found to be the lowest and highest for Tabor *var.* (−23.1 mV) and Fatima *var.* (−30.7 mV), respectively. As for the seeds grown in Melita, MB, the surface charge was the lowest and highest for concentrates produced from Fatima *var.* (−19.4 mV) and FB 18-20 *var.* (−31.8 mV), respectively. The effects of genotype and environment on hydrophobicity were not significant (Table 3). The values of hydrophobicity averaged for all protein concentrates at 8.5 arbitrary units (Table 5).

The ability of a protein ingredient to lower the interfacial or surface tension at the oil–water or air–water interface is critical to form stable emulsions and foams, respectively. The effect of environment on the interfacial tension was highly significant ($p < 0.001$); however, the effect of genotype and the interaction effect between genotype and environment were not (Table 3). The interfacial tension for protein concentrates (independent of genotype) was found

Table 4 Amino acid composition of faba bean protein concentrates as a function of genotype (Morden, MB, from one processing run)

Amino acids	SSNS-1	Taboar	Fatima	FB 9-4	FB 18-20
Neutral AA					
Glycine (%)	2.58	2.58	2.81	2.38	2.39
Alanine (%)	2.49	2.57	2.8	2.41	2.43
Serine (%)	3.31	3.28	3.59	3.07	3.11
Threonine (%)	2.22	2.37	2.5	2.08	2.24
Cysteine (%)	0.68	0.71	0.54	0.72	0.70
Subtotal	11.28	11.51	12.24	10.66	10.87
Charged AA					
Aspartic Acid + Asparagine (%)	6.55	6.77	7.35	6.36	6.56
Glutamic Acid + Glutamine (%)	10.11	10.55	11.45	9.92	9.88
Histidine (%)	1.85	1.81	2.09	1.52	1.73
Arginine (%)	6.88	7.14	7.65	7.12	6.44
Lysine (%)	4.16	3.82	4.76	4.23	3.24
Subtotal	29.55	30.09	33.3	29.15	27.85
Hydrophobic AA					
Proline (%)	2.58	2.74	2.99	2.51	2.48
Tyrosine (%)	2.25	2.22	2.48	2.19	2.07
Valine (%)	2.61	2.58	2.95	2.52	2.36
Methionine (%)	0.37	0.41	0.32	0.40	0.41
Isoleucine (%)	2.49	2.23	2.72	2.32	2.01
Leucine (%)	4.84	4.62	5.44	4.63	4.00
Phenylalanine (%)	2.85	2.65	3.18	2.81	2.26
Tryptophan (%)	0.57	0.61	0.61	0.58	0.57
Subtotal	18.56	18.06	20.69	17.96	16.16
Total amino acids (%)	59.39	59.67	66.22	57.76	54.89

to be lower for those produced from seeds grown in Melita (7.9 mN/m) than those grown in Morden (10.2 mN/m) (Table 5). No significant effects were found for the surface tension in response to either genotype or environment (Table 3), having an average value of 46.2 mN/m (Table 5).

Functionality of faba bean protein concentrates as a function of genotype and environment

The effects of genotype and environment on faba bean protein concentrates' foam-forming abilities (foaming capacity) were found to be insignificant (Table 3). The values ranged between 122 and 154 % (Table 6). The foam stability was found to be influenced by the environment and by the interaction between genotype and environment ($p < 0.05$); the main effect of genotype was not significant (Table 3). For protein concentrates produced from breeder seeds grown in Morden, MB, the foam stability was found to be the lowest for FB 9-4 *var*. (72 %) and highest for FB 18-20 *var*. (80 %). As for the concentrates produced from seeds grown in Melita, MB, FB 18-20 *var*. (71 %) had the lowest and FB 9-4 *var*. (73 %) had the highest foam stability (Table 6). The foaming capacity of the faba bean protein concentrates from breeder seeds (~140 %, Table 6) was similar to that of the commercial soy ingredient (137 %), but was much lower than that of the commercial faba bean (179 %), pea (208 %) and Great Northern bean (257 %) (Table 7). However, the foam stability of the faba bean protein concentrates produced from breeder seeds (73 %, Table 6) was similar to that of the soy (70 %) and Great Northern bean (71 %) protein concentrates and higher than that of the commercial faba bean (64 %) and pea (63 %) protein concentrates (Table 7).

Table 5 Surface properties (zeta potential, surface hydrophobicity, interfacial tension and surface tension) of faba bean protein concentrates as a function of genotype and environment (Morden vs. Melita, MB)

Genotype	Zeta potential (mV)		Surface hydrophobicity (a.u.)	
	Morden	Melita	Morden	Melita
SSNS-1	−28.7 ± 4.7	−28.1 ± 3.2	7.68 ± 0.93	8.82 ± 1.24
Taboar	−23.1 ± 1.7	−22.7 ± 1.2	6.89 ± 0.42	6.91 ± 0.85
Fatima	−30.7 ± 0.3	−19.4 ± 0.3	8.65 ± 3.08	9.33 ± 0.08
FB 9-4	−27.6 ± 3.2	−26.1 ± 2.7	6.84 ± 2.73	8.94 ± 1.47
FB 18-20	−27.7 ± 1.3	−31.8 ± 0.2	11.21 ± 0.80	10.29 ± 0.80
Genotype	Interfacial tension (mN/m)		Surface tension (mN/m)	
	Morden	Melita	Morden	Melita
SSNS-1	10.5 ± 0.2	7.2 ± 1.3	47.6 ± 0.4	44.3 ± 1.7
Taboar	10.2 ± 0.5	6.3 ± 1.8	45.0 ± 1.0	47.2 ± 2.6
Fatima	10.5 ± 0.0	9.1 ± 0.2	47.4 ± 0.5	46.3 ± 0.1
FB 9-4	9.2 ± 1.1	8.6 ± 0.1	45.0 ± 0.3	46.7 ± 0.8
FB 18-20	10.6 ± 0.1	8.3 ± 0.1	46.6 ± 1.4	46.6 ± 0.1

Data represent mean values ± one standard deviation from duplicate processing runs ($n = 2$)

Table 6 Functional properties of faba bean protein concentrates as a function of genotype and environment (Morden vs. Melita, MB)

Genotype	Foaming capacity (%)		Foam stability (%)	
	Morden	Melita	Morden	Melita
SSNS-1	151 ± 18	136 ± 7	73 ± 1	72 ± 0
Taboar	147 ± 3	142 ± 4	72 ± 4	72 ± 2
Fatima	139 ± 4	134 ± 8	73 ± 1	73 ± 1
FB 9-4	123 ± 16	154 ± 3	72 ± 1	73 ± 0
FB 18-20	150 ± 5	122 ± 7	80 ± 2	71 ± 0
Genotype	Emulsion stability (%)		Oil-holding capacity (g/g)	
	Morden	Melita	Morden	Melita
SSNS-1	83 ± 4	90 ± 13	1.1 ± 0.0	1.2 ± 0.0
Taboar	74 ± 8	83 ± 1	1.1 ± 0.0	1.2 ± 0.1
Fatima	78 ± 3	92 ± 4	1.1 ± 0.0	1.3 ± 0.2
FB 9-4	84 ± 1	82 ± 1	1.2 ± 0.1	1.1 ± 0.0
FB 18-20	85 ± 11	82 ± 4	1.2 ± 0.1	1.1 ± 0.0
Genotype	Water hydration capacity (g/g)		Solubility (%)	
	Morden	Melita	Morden	Melita
SSNS-1	0.5 ± 0.0	0.5 ± 0.0	84.9 ± 0.9	87.9 ± 0.5
Taboar	0.6 ± 0.0	0.5 ± 0.0	83.6 ± 0.1	87.7 ± 1.2
Fatima	0.5 ± 0.0	0.5 ± 0.0	88.0 ± 3.0	82.3 ± 0.7
FB 9-4	0.6 ± 0.1	0.6 ± 0.1	85.5 ± 0.9	87.9 ± 2.6
FB 18-20	0.6 ± 0.0	0.5 ± 0.0	85.8 ± 0.3	86.8 ± 1.7

Data represent mean values ± one standard deviation from duplicate processing runs ($n = 2$)

Table 7 Functional properties of commercial protein concentrates

Commercial protein concentrates	Foaming capacity (%)	Foam stability (%)	Emulsion stability (%)	Oil-holding capacity (g/g)	Water hydration capacity (g/g)	Solubility (%)
Faba Bean	179 ± 3	64 ± 2	92 ± 2	1.19 ± 0.07	0.87 ± 0.01	91 ± 4
Pea	208 ± 2	63 ± 2	92 ± 1	1.15 ± 0.01	0.75 ± 0.03	92 ± 3
Soy	137 ± 14	70 ± 3	89 ± 7	1.29 ± 0.04	4.96 ± 0.02	22 ± 0
Great Northern Bean	257 ± 10	71 ± 2	96 ± 2	1.14 ± 0.04	3.00 ± 0.15	95 ± 3

The effects of genotype and environment on the emulsion stability, oil-holding capacity and water hydration capacity were found to be insignificant (Table 3). The emulsion stability of the faba bean protein concentrates produced from breeder seeds was ~83 % (ranged between 74 and 90 %), which was lower than that of all commercial protein concentrates tested. The Great Northern bean's protein concentrate was found to have the highest emulsion stability (96 %), followed by the commercial faba bean/pea (92 %) and then the soy (89 %) (Table 7). Oil-holding capacity of the faba bean protein concentrates produced from breeder seeds was 1.2 g/g (ranged between 1.1 and 1.3 g/g), which was similar to all commercial concentrates: Great Northern bean (1.1 g/g), pea (1.1 g/g), faba bean (1.2 g/g) and soy (1.3 g/g) (Table 7). Water hydration capacity of the faba bean protein concentrate produced from breeder seeds was 0.5 g/g (ranging between 0.5 and 0.6 g/g) (Table 6), which was substantially lower than that of soy (5.0 g/g) and Great Northern bean (3.0 g/g), but only slightly lower than the commercial faba bean (0.9 g/g) and pea (0.7) concentrates (Table 7).

As for the protein concentrates' solubility, the effect of genotype and the interaction between genotype and environment were significant, whereas the main effect of environment was not (Table 3). Depending on the genotype and the environment, the values of solubility were found to range between ~82 and 88 %. In Morden, MB, *var.* Fatima had the highest solubility (88 %), whereas in Melita, MB,

var. SSNS-1 and FB 9-4 had the highest values (Table 6). The solubility of the faba bean protein concentrates produced from breeder seeds was slightly lower in comparison with the commercial ones, except for the soy, which had the lowest value within all samples (Table 7). The Great Northern bean's protein concentrate was found to have the highest solubility (95 %), followed by the commercial pea (92 %) and then the faba bean (91 %).

Conclusions

In the present study, the effect of genotype and environment on the ratio of legumin to vicilin proteins (*L*/*V*), the surface characteristics and the functionality of air-classified faba bean protein concentrates were assessed. The effect of environment significantly influenced the *L*/*V* ratio, interfacial tension and foam stability, whereas genotype affected the surface charge and solubility of the protein concentrates. The interaction effect between genotype and environment was significant for zeta potential, foam stability and protein concentrates' solubility. The functional attributes of the faba bean protein concentrates were compared with a number of commercial concentrate ingredients. The foaming capacity was similar to that of the commercial soy ingredient, whereas the foam stability was higher than the commercial faba bean and pea protein concentrates. The Great Northern bean protein concentrate was found to have the highest emulsion stability. Oil-holding capacity of the faba bean protein concentrates was similar to all commercial concentrates. Water hydration capacity was substantially lower than that of soy and Great Northern bean, but similar to the commercial faba bean and pea concentrates. The solubility of the faba bean protein concentrates was slightly lower in comparison with the commercial ones, except for the soy, which had the lowest value within all samples. The Great Northern bean protein concentrate was found to have the highest solubility, followed by the commercial pea.

In summary, although genotype x environment effects were evident among the faba bean protein concentrates, differences were not substantial enough to warrant variety screening and separation prior to secondary processing. However, it is important to note that this study did not account for nutritional aspects of the faba bean. Breeding programs today are focusing on lowering levels of the anti-nutritional factors in certain varieties to improve their nutrition.

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Compliance with ethical standards

Conflict of interest None.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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