Effect of genotype on the physicochemical and functional attributes of faba bean (*Vicia faba* L.) protein isolates

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Abstract The functionality and surface characteristics of protein isolates prepared from seven genotypes of faba bean were investigated. All factors studied were independent of genotype. The average protein and isolate (~94% protein) yields were ~77% and ~25%, respectively, using an alkaline extraction process followed by isoelectric precipitation. The overall averages were: surface charge +22.1 mV, surface hydrophobicity 47.2 arbitrary units, and surface and interfacial tensions of 65.0 mN/m and 10.7 mN/m, respectively. The ratio of the major globulin fractions (legumin:vicilin) shifted from 3.8 for the flours to 4.5 in the isolates. Average values for foaming capacity and stability, emulsion capacity, creaming stability, oil holding capacity, emulsifying activity and stability indices, and solubility were 162.0%, 65.0%, 184.0 g/g, 94.0%, 5.7 g/g, 13.0 m²/g, 10.7 min, and 81.0%, respectively. The lack of significant varietal effects would be advantageous to food processors as the source of the feedstock would not affect ingredient functionality.

Keywords: faba bean, protein isolates, genotype, functionality, legumin:vicilin ratio

Introduction

In human food, proteins from various sources are consumed for their nutritional and health-promoting benefits. However, in recent times, the food industry has been leaning toward plant-based alternatives in the formulation of food products because of “increasing dietary preferences among consumers, growing cost difference between animal and plant proteins, and rising concerns over the safe consumption of animal-based products” (1). Pulses represent a good source of protein (>20 g protein/100 g dry matter), with protein levels being significantly higher than those of cereal grains or root crops (2). Pulses can be used in many ways, either through the direct consumption of regional crops including chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), and faba bean (*Vicia faba* L.), which is a frequent practice in developing countries, or as fractions (flours, protein concentrates, and protein isolates) in prepared or processed foods. Faba bean (also known as broad bean when used in vegetable form) is widely grown in countries such as China, India, and Pakistan (3), and has a high protein level (32.5% on a dry weight basis) (4). It has a well-balanced amino acid composition when consumed with cereals (5) and is a good source of vitamins, minerals, and fiber (6). However, its use in the form of a protein isolate is very limited, partially because of the poor understanding of structure–function relationships, a decrease in production of faba bean in recent years,

and the presence of anti-nutritional compounds such as glycosides, which are reported to cause favism, a severe form of anemia, in some people (7,8).

The major storage proteins found in pulses are the salt-soluble globulins, which include legumin (L) (11S, S=Svedberg unit; molecular mass of ~350–400 kDa), vicilin (V) (7S; molecular mass of ~150 kDa), and convicilin (7-8S; molecular mass of ~270 kDa). The legumin:vicilin (L/V) ratio is not fixed and varies among cultivars (9–11). Variability in the L/V ratio may be associated with various intrinsic and extrinsic factors. Intrinsic factors include seed coat type (wrinkled or smooth), flower color, seed color, and seed size (12,13), whereas extrinsic factors comprise agronomic factors and the method of analysis (14). Variation in the L/V ratio may influence the physicochemical and functional properties of pulse protein products (15–16). For instance, Bora *et al.* (17) observed that 7S pea globulins underwent heat gelation, whereas 11S globulins did not under the same processing conditions. Barac *et al.* (18) reported that pea genotypes with high 7S protein levels or low 11S protein levels yielded higher amounts of protein (higher protein extractability) compared with other genotypes. Mertens *et al.* (14) reported significant differences in protein content and L/V ratio for different genotypes of smooth pea, locations, and years of growth. However, limited work has been done describing the impact of environmental factors on the nutritional value and functionality of faba bean or the structure–function relationships

between L/V ratio and functional attributes.

The overall aim of this research was to investigate structure–function relationships determining the functionality of faba bean protein isolates with respect to genotype. Seven genotypes (CDC Fatima, Taboar, SSNS-1, FB9-4, FB18-20, Snowbird, and CDC Snowdrop) grown in 2011 and 2012 from two field plots at various locations in Western Canada were assessed. These genotypes were selected based on their physical and chemical characteristics such as tannin level, seed size, and color. For instance, both Snowbird and CDC Snowdrop are zero-tannin genotypes having seeds with seed coats that are very light in color with no redness, in contrast to tannin-containing genotypes that are darker in color with a redness character. It was hypothesized that genotypic differences in the L/V ratio would be a significant determinant of the functionality (e.g., solubility, foaming capacity and stability, emulsion capacity, creaming stability, emulsification activity and stability indices, and oil holding capacity) of faba bean protein isolates. Confirmation of this hypothesis might make possible the selection of genotypes or production lots particularly suitable for producing isolates that would be competitive in the plant protein market.

Materials and Methods

Materials Seed samples from seven faba bean (*Vicia faba* L.) genotypes (CDC Fatima, Taboar, SSNS-1, FB9-4, FB18-20, Snowbird, and CDC Snowdrop) grown in 2011 and 2012, each from two field plots across Western Canada were kindly donated by the Crop Development Centre at the University of Saskatchewan (Saskatoon, SK, Canada). All genotypes had normal vicine levels. Snowbird and CDC Snowdrop were the only zero-tannin genotypes. SSNS-1 and CDC Snowdrop were of small seed size; Taboar, Fatima, and Snowbird were of intermediate seed size; and FB18-20 and FB9-4 were of large seed size. Snowbird and CDC Snowdrop seeds were light in color with no redness, while FB9-4, FB18-20, and CDC Fatima seeds were darker in color with slight redness; the SSNS-1 and Taboar seeds were dark in color with high redness.

In this study, the following commercial isolates were employed for comparative purposes: Propulse pea protein isolate (Nutri-Pea Limited, Portage La Prairie, MB, Canada), dried egg white (Ballas Egg Product Corp., Zanesville, OH, USA), Prolisse soy protein isolate (Cargill, Wayzata, MN, USA), PROLITE 100 wheat protein isolate (Archer Daniels Midland Co., Decatur, IL, USA), and whey protein isolate (BiPro, Eden Prairie, MN, USA). All chemicals used in this study were of reagent grade except for sodium dodecyl sulfate, Tris, and glycine, which were of electrophoresis purity. 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 protein isolates Faba bean seeds were dehulled using a Satake mill (Satake, Penrith, NSW, Australia), ground

into coarse flour using a disc mill (Glen Mills Inc., Clifton, NJ, USA), and then ground into finer flour using a UDY Cyclone Sample Mill (UDY Corp., Fort Collins, CO, USA). All flours were defatted according to Can Karaca *et al.* (16) with slight modifications. In brief, each flour sample (~400 g) was mixed with hexane (1:3, w/v, ~1200 mL) and stirred for 40 min using a mechanical stirrer (500 rpm) within a fume hood. Hexane was decanted, and the process was repeated two more times. The resultant hexane-flour mixture was then filtered using Whatman #1 filter paper (Whatman International Ltd., Maidstone, UK) followed by drying overnight in a fume hood. The dried and defatted flour was stored at 4°C until used. Faba bean protein isolates were prepared by alkaline extraction followed by isoelectric point precipitation according to Makri *et al.* (19). In brief, 350–400 g of defatted flour was dispersed in deionized water (1:10, w/v) and adjusted to pH 9.5 with 1 M NaOH, followed by continuous stirring (500 rpm) at room temperature (21–23°C) for 40 min and subsequent centrifugation (1,600× *g*, 20 min, 4°C) using a Sorvall RC-6 Plus centrifuge (Thermo Scientific, Waltham, MA, USA). The supernatant was collected, and the pellet was re-suspended in water (1:5, w/v), stirred, and then centrifuged (1,600× *g*, 20 min, 4°C). The supernatants were combined, the pH adjusted to 4.5 using 1 M HCl, and then centrifuged (1,600× *g*, 20 min, 4°C). The recovered pellet was suspended in deionized water, adjusted to pH 7.0 using 1 M NaOH, stored at 30°C, and later freeze dried to obtain the protein isolate. Proximate analysis of all flour materials was performed according to Association of Official Analytical Chemists methods 925.10, 923.03, and 997.09 for moisture, ash, and crude protein (%N×6.25), respectively (20). Crude fat levels were considered negligible because of the prior defatting step. All proximate analyses were reported as mean ± one standard deviation (*n*=2).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions was performed for all flour and isolate samples to identify the relative proportions of legumin and vicilin (reported as the legumin:vicilin (L/V) ratio). Legumin and vicilins fractions were separated via SDS–PAGE according to Laemmli (21) with some modifications. In brief, 75 µL of a 3 mg/mL flour–water (or 1 mg/mL isolate–water) suspension was diluted with 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, Hercules, CA, USA) 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 model 5424 centrifuge (Eppendorf, Hamburg, Germany) at 12,000× *g* for 5 min. Precast 4–20% Precise Tris–glycine gels (Thermo Scientific) were used to run the samples in a MG–V202 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 weight markers. Gels were then stained using Phastgel Blue R tablets (GE Healthcare Life Sciences, Uppsala, Sweden) for 1 h, followed by de-staining with methanol:water:acetic acid (3:6:1 v/v/v) three times over a 24-h period. The de-stained gels were then scanned using an EPSON Perfection V750 Pro scanner (EPSON, Shinjuku, Tokyo). Bands from the SDS-PAGE analysis were quantified by implied densitometry using Image J software (National Institutes of Health, Bethesda, MD, USA) (22). All samples were run in triplicate. The L/V ratio was reported as the mean ± standard deviation ($n=3$).

Physicochemical and functional properties For assessment of physicochemical and functional properties, protein isolate solutions of various concentrations were prepared by dispersing the isolate powder (at concentrations corrected for protein weight on a moisture-free basis) in 10 mM sodium phosphate buffer (pH 7.0) (w/w), followed by overnight stirring (500 rpm, 4°C, ~16 h). All measurements are reported as the mean ± standard deviation ($n=2$).

Physicochemical properties: Surface charge (i.e., zeta potential) was measured for 0.05% (w/w) protein solutions (i.e., 0.05 g protein within 100 g water or 0.053 g isolate (93.9% protein, dry basis)) 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:

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

where ε is permeability (F (Farad)/m), $f(k\alpha)$ is a function associated with the ratio of the 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.

Intrinsic fluorescence of 0.05% (w/w) protein solutions was measured to provide an estimate of surface hydrophobicity using a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA). The samples were excited at a fixed wavelength of 295 nm with a slit width of 2.5 nm, whereas the emission wavelength was set at 285–450 nm, in 0.5 nm increments, with a slit width of 5 nm. Fluorescence intensity (FI) was measured as a function of the emission wavelength (nm) and had the spectrum of the buffer (10 mM sodium phosphate buffer, pH 7.0) removed. The maximum FI values of the resulting spectra were recorded.

Interfacial tension between 0.25% (w/w) protein solutions and canola oil, and between protein solutions and the air interface, were determined using a Du Noüy ring and a semi-automatic 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) was calculated from the following equation using the maximum force ($F_{\max}$)

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

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

Functional properties: Oil holding capacity (OHC) was determined according to Chakraborty (23) with slight modifications. Ten milliliters of canola oil was combined with 1 g of protein (or 1.06 g isolate) in a weighed centrifuge tube. The mixture was vortexed for 10 s every 5 min for a total duration of 30 min, followed by decanting of the supernatant. The OHC was calculated according to Eq. (3),

$$OHC = \frac{Wt_{Wet} - Wt_{Dry}}{Wt_{Dry}} \times 100\%, \quad (3)$$

where Wt_{Wet} and Wt_{Dry} are the weights of the wet and dry samples, respectively.

Foaming capacity (FC) and foaming stability (FS) for 1.0% (w/w) protein solutions were determined according to Liu *et al.* (1). Fifteen milliliters of each protein solution was transferred to a 400 mL beaker for homogenization using a Macro Homogenizer (Omni International, Marietta, GA, USA) equipped with a 20 mm saw tooth probe at speed 4 (~7,200 rpm) for 5 min. The homogenized sample was then transferred immediately to a 100 mL graduated cylinder, and the foam volume was measured at 0 and 30 min, with FC and FS determined using Eq. (4) and (5), respectively.

$$FC = \frac{V_{F0}}{V_{sample}} \times 100\%, \quad (4)$$

$$FS = \frac{V_{F30}}{V_{F0}} \times 100\%, \quad (5)$$

where V_{F0} is the volume of the foam at 0 min, V_{sample} is the initial volume of sample used (15 mL), and V_{F30} is the foam volume after 30 min.

Emulsion capacity was determined according to Can Karaca *et al.* (16) with slight modifications. Two grams of a 1.0% (w/w) protein solution was homogenized with various quantities of canola oil (2–5 g) using a Macro Homogenizer (Omni International) equipped with a 20 mm saw tooth probe at speed 4 (~7,200 rpm) for 5 min. The conductivity of the emulsion was measured using an Orion 3-Star bench top conductivity meter (Thermo Scientific) with a 4-electrode conductivity cell until an inversion point was observed (a sharp drop in conductivity indicating inversion of the oil-in-water emulsion to water-in-oil). Emulsion capacity (g oil/g protein) was expressed as the average of the amount of oil homogenized (g) per gram of protein before and after the inversion point.

Creaming stability (CS) of 1.0% (w/w) protein solutions was measured by homogenizing 5 mL of protein solution with 5 mL of canola oil using a Macro Homogenizer (Omni International) equipped with a 20 mm saw tooth probe at speed 4 (~7,200 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 creaming stability was calculated using Eq. (6).

$$CS = \frac{V_B - V_A}{V_A} \times 100\%, \quad (6)$$

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.

Emulsion activity (EAI) and emulsion stability (ESI) indices for 0.5% (w/w) protein solutions were determined according to the method of Pearce and Kinsella (24). Fixed quantities of protein isolate solution (5 g) and canola oil (5 g) were homogenized using a Macro Homogenizer (Omni International) equipped with a 20 mm saw tooth probe at speed 4 (~7,200 rpm) for 5 min. Immediately after homogenization, a 50 μ L aliquot of emulsion was taken from the bottom of the tube ($t=0$ min) and diluted with 7.5 mL of 0.1% SDS in 10 mM sodium phosphate buffer (pH 7.0). The diluted emulsion was vortexed for 10 s and then measured for absorbance at 500 nm using a Genesys 10 UV-visible spectrophotometer (Thermo Scientific) using plastic cuvettes (1 cm path length). Similarly, another 50 μ L aliquot of emulsion was taken after 10 min, and the absorbance was measured using the procedure described above. EAI and ESI were calculated using the following equations.

$$EAI = \frac{2 \times 2.203 \times A_0 \times N}{c \times \varphi \times 10000}, \quad (7)$$

$$ESI = \frac{A_0}{\Delta A} \times t, \quad (8)$$

where EAI (m^2/g) is expressed as the area of interface (m^2) stabilized per unit weight of protein (g) and ESI is expressed in minutes; A_0 represents the absorbance of the diluted emulsion immediately after homogenization, N is the dilution factor, c is the concentration of the protein solution (g/mL), and φ is the oil volume fraction. ΔA ($A_0 - A_{10}$) is the absorbance difference between samples taken at 0 min and 10 min, and t is the time interval (10 min).

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 isolate solution was transferred to a 50 mL centrifuge tube followed by centrifugation ($9,100 \times g$, 10 min, 4°C) using a Sorvall RC-6 Plus Superspeed Centrifuge (Thermo Scientific). Then, 15 g of the supernatant obtained was taken (measured using an analytical balance) for determination of the crude 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 isolate 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%.

Table 1. Protein, ash, and legumin: vicilin ratios levels in faba bean protein isolates produced from different genotypes, years, and locations

	Protein (%, d.b.)	Ash (%, d.b.)	Lg/Vn ratio
a) CDC Fatima			
Arborg, MB (2011)	96.56 $\pm$ 0.11	5.02 $\pm$ 0.09	4.51 $\pm$ 0.13
Lacombe, AB (2011)	93.86 $\pm$ 0.02	6.01 $\pm$ 0.16	4.41 $\pm$ 0.02
Outlook, SK (2012)	94.90 $\pm$ 0.06	5.28 $\pm$ 0.04	5.08 $\pm$ 0.19
Saskatoon-Preston, SK (2012)	93.83 $\pm$ 0.00	5.93 $\pm$ 0.04	4.61 $\pm$ 0.22
b) Taboar			
Arborg, MB (2011)	94.43 $\pm$ 0.16	6.09 $\pm$ 0.53	3.79 $\pm$ 0.09
Lacombe, AB (2011)	95.34 $\pm$ 0.16	5.30 $\pm$ 0.33	4.26 $\pm$ 0.11
Outlook, SK (2012)	93.70 $\pm$ 0.25	5.99 $\pm$ 0.33	4.94 $\pm$ 0.10
Saskatoon-Preston, SK (2012)	94.34 $\pm$ 0.03	5.86 $\pm$ 0.15	3.99 $\pm$ 0.18
c) SSNS-1			
Arborg, MB (2011)	94.49 $\pm$ 0.06	4.85 $\pm$ 0.32	5.06 $\pm$ 0.11
Edmonton, AB (2011)	93.48 $\pm$ 0.09	5.94 $\pm$ 0.06	3.91 $\pm$ 0.11
Outlook, SK (2012)	99.36 $\pm$ 0.11	5.85 $\pm$ 0.13	4.63 $\pm$ 0.15
Rosthern, SK (2012)	92.95 $\pm$ 0.19	5.32 $\pm$ 0.21	5.13 $\pm$ 0.23
d) FB 9-4			
Arborg, MB (2011)	94.82 $\pm$ 0.17	5.49 $\pm$ 0.03	3.50 $\pm$ 0.26
Edmonton, AB (2011)	92.80 $\pm$ 0.32	5.50 $\pm$ 0.15	5.65 $\pm$ 0.24
Outlook, SK (2012)	93.18 $\pm$ 0.00	6.04 $\pm$ 0.11	5.56 $\pm$ 0.35
Rosthern, SK (2012)	94.12 $\pm$ 1.24	5.09 $\pm$ 0.01	4.81 $\pm$ 0.20
e) FB 18-20			
Arborg, MB (2011)	93.10 $\pm$ 0.36	5.60 $\pm$ 0.03	4.30 $\pm$ 0.28
Edmonton, AB (2011)	93.04 $\pm$ 0.24	6.26 $\pm$ 0.56	4.78 $\pm$ 0.26
Outlook, SK (2012)	93.53 $\pm$ 0.38	6.20 $\pm$ 0.18	5.78 $\pm$ 0.06
Rosthern, SK (2012)	92.65 $\pm$ 0.03	5.92 $\pm$ 0.09	4.16 $\pm$ 0.16
f) Snowbird			
Lacombe, AB (2011)	94.87 $\pm$ 0.34	5.20 $\pm$ 0.11	3.96 $\pm$ 0.28
Melita, MB (2011)	92.14 $\pm$ 0.08	6.88 $\pm$ 0.30	3.91 $\pm$ 0.24
Outlook, SK (2012)	94.70 $\pm$ 0.19	6.21 $\pm$ 0.07	4.08 $\pm$ 0.06
Saskatoon-Sutherland, SK (2012)	93.56 $\pm$ 0.30	5.85 $\pm$ 0.06	4.00 $\pm$ 0.42
g) Snowdrop			
Edmonton, AB (2011)	95.07 $\pm$ 0.06	5.78 $\pm$ 0.03	4.46 $\pm$ 0.22
Saskatoon-Sutherland, SK (2011)	90.54 $\pm$ 0.08	6.36 $\pm$ 0.30	4.80 $\pm$ 0.15
Rosthern, SK (2012)	93.33 $\pm$ 0.04	5.79 $\pm$ 0.13	3.74 $\pm$ 0.27
Meath Park, SK (2012)	91.28 $\pm$ 0.03	6.72 $\pm$ 0.49	3.25 $\pm$ 0.15

Data represent the mean $\pm$ standard deviation ($n=2$).

Statistical methods A one-way analysis of variance (ANOVA) along with a post-hoc Tukey test was performed to evaluate the effect of genotype for all physicochemical and functional properties using SPSS software (IBM Corporation, Armonk, NY, USA). A simple Pearson correlation (r) analysis was performed to identify any significant correlations among the compositional, physicochemical, and functional properties of the faba bean protein isolates.

Results and Discussion

Composition of isolates Faba bean protein isolates were prepared from defatted flours using an alkaline extraction process followed by isoelectric precipitation. Process efficiency was described by both the

Fig. 1. Representative examples of A) SDS-PAGE gel for isolate (Ai) and flour (Aii) prepared from FB18-20; and (B) a corresponding densitometry analysis for the isolate protein profile.

isolate yield and protein yield. The former is a measure of the amount of isolate obtained relative to the amount of flour used in the extraction process, whereas the latter is a measure of the amount of protein present in the isolate relative to the amount of protein present in the flour used to prepare the isolate. A one-way ANOVA revealed no significant differences in isolate or protein yield because of genotype ($p > 0.05$). Average isolate and protein yields were $25.3 \pm 2.1\%$ and $76.6 \pm 5.4\%$, respectively. A one-way ANOVA also revealed that the effects of genotype on the protein and ash contents of protein isolates were non-significant ($p > 0.05$). Average protein and ash levels in the isolates, on a moisture-free basis, were $93.9 \pm 1.6\%$ and $5.8 \pm 0.5\%$, respectively (Table 1).

Using densitometry, the relative percentages of the legumin (L) [α -legumin chain (37 kDa) + β -legumin chain (20 kDa)] and vicilin (V) [50 kDa] were determined and reported as the L/V ratio. A representative SDS-PAGE gel for an isolate (Fig. 1Ai) and a flour (Fig. 1Aii) prepared from genotype FB18-20 is presented, along with the corresponding densitometry analysis for the isolate only (Fig. 1B). Band identification was based on published values from Nikolic *et al.*

(25) and Tucci *et al.* (26). For the protein isolate, four major bands were identified corresponding to molecular masses of ~73, ~51, ~34, and ~21 kDa, and were presumed to correspond to convicillin, vicilin, α -legumin, and β -legumin, respectively. Similarly, for the corresponding flour sample, four major bands were identified corresponding to molecular masses of ~73, ~53, ~36, and ~21 kDa, again representing convicillin, vicilin, α -legumin, and β -legumin, respectively. Minor (faint) bands were not included in the analysis, and might represent albumin storage proteins and/or metabolic proteins, e.g., enzymes. The extraction process used in the preparation of protein isolates from flour eliminated some bands. The L/V ratio was similar in magnitude across all genotypes for both faba bean flours (range = 3.4–4.6, average = 3.8) and isolates (range = 4.0–4.9, average = 4.5) ($p > 0.05$) (Table 1). Gatehouse *et al.* (9) reported the L/V ratio to range between 2.1 and 3.6 for isolates produced using a salt extraction process, which was slightly lower than that in the present study, possibly because of varietal differences, the environment, or processing conditions (salt extraction vs. alkaline extraction-isoelectric precipitation).

Physicochemical properties Both the surface charge and hydrophobicity of a protein are important characteristics underlying how well a protein remains in solution or interacts with an apolar interface in a foam or an emulsion. If the zeta potential is high (approximately +30 mV), particles tend to repel one another in solution because of electrostatic repulsive forces resulting in greater solubility, whereas at lower values, instability occurs as proteins tend to aggregate. Surface hydrophobicity relates to the amount of aromatic amino acid residues (e.g., tryptophan, tyrosine, and phenylalanine) exposed at the surface. In the present study, differences in zeta potential and hydrophobicity (intrinsic fluorescence) among isolates were not significant across genotypes, with an average potential of +22.1 mV and 47.2 arbitrary units, respectively, at pH 7.0 ($p>0.05$) (Table 2). A Pearson correlation analysis was performed to observe whether any relationships existed between the L/V ratios of the isolates and the physicochemical and functional

properties. The L/V ratio of the isolate was found to be negatively correlated with zeta potential ($r=0.39$, $p<0.05$) and positively correlated with surface hydrophobicity ($r=0.40$, $p<0.05$), indicating that the isolate becomes less charged as the legumin content increases.

During foam or emulsion generation, proteins migrate to the air–water or oil–water interfaces where they unravel and re-orient such that hydrophobic moieties face toward the oil or air phase, and hydrophilic moieties are oriented toward the polar, aqueous phase (27). Proteins then interact to form a viscoelastic film surrounding a lipid droplet or gas bubble (28). The process acts to lower the interfacial forces between the two phases to produce smaller, more stable droplets or bubbles. In the present study, the ability of the isolates to lower surface and interfacial tension was similar across genotypes, lowering it from 95.3 to 26.2 mN/m and from 65.0 to 10.7 mN/m ($p<0.05$) (Table 2).

Table 2. Surface and interfacial properties for faba bean protein isolates produced from different genotypes, years, and locations

Genotype	Surface charge (mV)	Hydrophobicity (au)	Surface tension (mN/m)	Interfacial tension (mN/m)
a) CDC Fatima				
Arborg, MB (2011)	23.20±0.99	46.80±1.70	63.51±1.70	11.10±0.57
Lacombe, AB (2011)	22.30±1.13	45.20±1.41	65.82±0.04	11.40±0.30
Outlook, SK (2012)	20.15±0.35	44.50±0.57	67.90±0.86	10.76±0.22
Saskatoon-Preston, SK (2012)	21.45±0.35	50.70±1.41	64.60±0.46	10.64±0.26
b) Taboar				
Arborg, MB (2011)	20.70±1.41	48.65±0.07	65.41±0.36	11.47±0.21
Lacombe, AB (2011)	21.95±0.50	43.65±1.34	65.14±1.87	10.68±0.06
Outlook, SK (2012)	22.00±0.14	48.25±0.07	67.90±2.52	11.59±0.54
Saskatoon-Preston, SK (2012)	20.50±1.41	44.80±2.40	64.97±1.17	10.61±0.02
c) SSNS1				
Arborg, MB (2011)	21.20±1.56	49.90±1.13	64.41±0.81	10.54±0.13
Edmonton, AB (2011)	23.10±0.14	43.30±0.28	67.72±2.43	11.21±0.49
Outlook, SK (2012)	20.30±1.13	47.25±0.78	63.95±0.73	10.74±0.16
Rosthern, SK (2012)	22.80±1.27	48.50±0.28	67.43±1.22	9.90±0.34
d) FB 9-4				
Arborg, MB (2011)	22.65±0.07	48.85±1.63	65.78±1.15	10.16±0.06
Edmonton, AB (2011)	22.05±0.64	53.40±1.56	66.58±0.71	11.39±0.04
Outlook, SK (2012)	21.55±0.35	50.20±0.42	64.85±0.83	10.48±0.31
Rosthern, SK (2012)	21.70±0.14	51.40±0.71	65.12±1.14	10.22±0.21
e) FB 18-20				
Arborg, MB (2011)	21.90±0.00	49.90±3.82	65.97±0.21	10.81±0.07
Edmonton, AB (2011)	20.25±0.21	36.85±1.91	63.05±2.05	10.77±1.05
Outlook, SK (2012)	21.15±0.92	50.55±2.90	63.0±1.36	10.98±0.28
Rosthern, SK (2012)	21.45±1.06	50.30±1.98	65.17±0.23	9.68±0.42
f) Snowbird				
Lacombe, AB (2011)	22.20±0.85	45.85±0.21	65.46±1.25	10.76±0.05
Melita, MB (2011)	24.40±0.85	45.45±0.35	62.45±0.72	10.90±1.05
Outlook, SK (2012)	24.90±0.42	50.00±0.57	63.29±0.63	9.92±0.28
Saskatoon-Sutherland, SK (2012)	22.35±0.78	44.35±1.91	63.56±1.78	10.15±0.42
g) Snowdrop				
Edmonton, AB (2011)	20.75±0.92	45.15±1.34	64.34±0.04	11.42±0.18
Saskatoon-Sutherland, SK (2011)	23.50±0.57	47.65±0.35	65.07±1.07	11.04±0.08
Rosthern, SK (2012)	23.55±1.20	47.10±1.41	63.39±0.04	10.07±0.04
Meath Park, SK (2012)	23.65±0.35	41.70±2.83	64.28±0.26	10.67±0.06

Data represent the mean±one standard deviation ($n=3$).

Functional properties The oil holding capacity for isolates prepared from faba bean was similar in magnitude across genotypes (average = 5.7 ± 0.2 g/g) ($p > 0.05$) (Table 3A). A high oil holding capacity in proteins is important industrially as a means of reducing lipid drainage during processing, and is related to a protein's emulsification capacity (29,30). In general, a greater number of non-polar amino acids available to bind oil results in higher oil holding capacities. For the faba bean isolates, however, no significant correlation was found between the oil holding capacity and surface hydrophobicity ($p < 0.05$). Values in the present study were higher than those reported by Fernandez-Quintela (31) (OHC ~ 1.8 g/g) and Vioque *et al.* (3) (OHC ~ 2.3 g/g) for faba bean protein isolates by isoelectric precipitation.

The solubility of all isolates was similar in magnitude across genotypes ($p > 0.05$) with a mean of $81.5 \pm 4.7\%$ (Table 3A). Similar solubility was reported by Can Karaca *et al.* (16). Good protein

solubility is important for the emulsification and foaming process to help facilitate their migration to the oil–water or air–water interface. In terms of emulsification, the emulsion capacity, creaming stability, emulsification activity, and stability indices were similar in magnitude across all genotypes, with averages of 184 ± 4.5 g/g, $94.0 \pm 1.8\%$, 13.0 ± 0.7 m²/g, and 10.7 ± 0.2 min, respectively (Table 3B). In foaming experiments, foam capacity and stability were found to be 162.0 ± 5.4 and $65.2 \pm 1.7\%$, respectively, irrespective of the genotype ($p > 0.05$) (Table 3A). Foam stability ($r = 0.54$, $p < 0.01$), cream stability ($r = 0.46$, $p < 0.05$), and solubility ($r = 0.44$, $p < 0.05$) were found to be positively correlated with zeta potential. Emulsion activity index and ESI were not significantly correlated with any physicochemical or functional properties.

Comparison to commercial protein isolates The functional properties of the faba bean protein isolates were compared with

Table 3A. Functional properties for faba bean protein isolates produced from different genotypes, years, and locations

Genotype	Oil holding capacity (g/g)	Solubility (%)	Foam capacity (%)	Foam stability (%)
a) CDC Fatima				
Arborg, MB (2011)	6.36 ± 0.03	91.36 ± 1.77	160.0 ± 9.4	63.52 ± 0.68
Lacombe, AB (2011)	5.29 ± 0.04	84.76 ± 1.00	160.0 ± 0.0	62.50 ± 0.00
Outlook, SK (2012)	6.31 ± 0.26	64.72 ± 1.76	156.7 ± 4.7	57.99 ± 0.99
Saskatoon-Preston, SK (2012)	4.94 ± 0.02	98.83 ± 0.94	155.0 ± 7.1	66.60 ± 3.04
b) Taboar				
Arborg, MB (2011)	4.91 ± 0.05	79.25 ± 0.74	175.0 ± 7.1	62.80 ± 2.85
Lacombe, AB (2011)	6.99 ± 0.01	65.79 ± 0.88	153.3 ± 0.0	68.53 ± 4.68
Outlook, SK (2012)	5.16 ± 0.07	81.86 ± 0.91	171.7 ± 7.1	67.02 ± 1.39
Saskatoon-Preston, SK (2012)	6.42 ± 0.11	72.12 ± 0.90	175.0 ± 7.1	56.21 ± 0.92
c) SSNS-1				
Arborg, MB (2011)	6.81 ± 0.62	87.90 ± 0.93	160.0 ± 0.0	61.46 ± 1.47
Edmonton, AB (2011)	5.41 ± 0.10	71.65 ± 1.88	143.3 ± 14.1	66.20 ± 1.69
Outlook, SK (2012)	6.62 ± 0.11	71.57 ± 0.88	170.0 ± 0.0	61.37 ± 1.94
Rosthern, SK (2012)	4.64 ± 0.10	95.91 ± 3.42	158.3 ± 2.4	71.59 ± 1.07
d) FB 9-4				
Arborg, MB (2011)	6.11 ± 0.49	76.91 ± 0.88	156.7 ± 4.7	61.68 ± 1.15
Edmonton, AB (2011)	4.91 ± 0.26	69.14 ± 1.92	150.0 ± 4.7	66.70 ± 2.10
Outlook, SK (2012)	5.76 ± 0.26	79.22 ± 0.96	158.3 ± 2.4	64.23 ± 2.44
Rosthern, SK (2012)	5.47 ± 0.62	79.85 ± 0.93	155.0 ± 2.4	68.83 ± 1.05
e) FB 18-20				
Arborg, MB (2011)	6.19 ± 0.02	92.23 ± 0.91	166.7 ± 0.0	61.00 ± 1.41
Edmonton, AB (2011)	6.40 ± 0.04	80.63 ± 0.93	160.0 ± 0.0	66.67 ± 2.95
Outlook, SK (2012)	6.27 ± 0.09	79.59 ± 1.76	160.0 ± 0.0	66.67 ± 0.00
Rosthern, SK (2012)	4.47 ± 0.21	87.51 ± 0.00	183.3 ± 4.7	68.19 ± 0.47
f) Snowbird				
Lacombe, AB (2011)	5.70 ± 0.53	76.82 ± 0.93	150.0 ± 9.4	68.83 ± 1.96
Melita, MB (2011)	5.21 ± 0.20	92.22 ± 0.98	175.0 ± 2.4	69.52 ± 0.41
Outlook, SK (2012)	6.00 ± 0.31	92.37 ± 1.87	150.0 ± 4.7	65.51 ± 2.66
Saskatoon-Sutherland, SK (2012)	5.80 ± 0.44	88.41 ± 0.93	163.3 ± 4.7	68.38 ± 0.53
g) Snowdrop				
Edmonton, AB (2011)	6.37 ± 0.01	70.72 ± 4.17	153.3 ± 0.0	55.90 ± 2.19
Saskatoon-Sutherland, SK (2011)	5.07 ± 0.15	82.35 ± 1.94	163.3 ± 4.7	67.33 ± 0.94
Rosthern, SK (2012)	4.90 ± 0.56	94.06 ± 0.00	168.3 ± 2.4	68.37 ± 7.96
Meath Park, SK (2012)	5.12 ± 0.21	73.91 ± 0.00	171.7 ± 7.1	70.91 ± 1.55

Data represent the mean $\pm$ one standard deviation ($n = 3$)

Table 3B. Functional properties for faba bean protein isolates produced from different genotypes, years, and locations

Genotype	Emulsion capacity (g/g)	Creaming stability (%)	Emulsion activity index (%)	Emulsion stability index (%)
a) CDC Fatima				
Arborg, MB (2011)	187.5±8.8	94.75±0.35	11.87±1.66	10.66±0.08
Lacombe, AB (2011)	187.5±8.8	96.00±0.00	12.84±0.61	10.75±0.06
Outlook, SK (2012)	187.5±8.8	95.00±1.41	11.40±1.19	10.76±0.22
Saskatoon-Preston, SK (2012)	193.8±0.0	95.50±0.71	12.45±0.14	10.62±0.05
b) Taboar				
Arborg, MB (2011)	181.3±0.0	92.00±2.83	11.83±0.12	10.66±0.14
Lacombe, AB (2011)	181.3±0.0	91.00±1.41	11.95±0.80	10.54±0.04
Outlook, SK (2012)	187.5±8.8	95.00±1.41	11.95±0.41	10.81±0.22
Saskatoon-Preston, SK (2012)	187.5±8.8	92.00±1.41	14.41±1.39	10.52±0.23
c) SSNS-1				
Arborg, MB (2011)	175.0±8.8	94.75±1.06	12.16±1.95	10.50±0.05
Edmonton, AB (2011)	181.3±0.0	95.00±0.00	13.56±0.06	10.64±0.25
Outlook, SK (2012)	175.0±8.8	94.50±0.00	15.77±0.33	10.53±0.05
Rosthern, SK (2012)	175.0±8.8	96.50±0.71	13.02±0.16	10.79±0.01
d) FB 9-4				
Arborg, MB (2011)	187.5±8.8	92.25±0.35	10.81±0.20	10.58±0.17
Edmonton, AB (2011)	175.0±8.8	94.00±0.00	13.67±0.49	10.68±0.12
Outlook, SK (2012)	181.3±0.0	90.00±0.00	12.17±1.70	10.60±0.23
Rosthern, SK (2012)	168.8±0.0	91.00±4.24	14.07±1.25	10.98±0.05
e) FB 18-20				
Arborg, MB (2011)	168.8±0.0	92.50±0.71	11.63±0.94	10.74±0.05
Edmonton, AB (2011)	181.3±0.0	91.00±1.41	12.84±1.93	10.70±0.48
Outlook, SK (2012)	200.0±8.8	89.75±2.47	14.58±0.72	11.03±0.38
Rosthern, SK (2012)	193.8±0.0	94.50±0.71	12.04±0.02	11.32±0.40
f) Snowbird				
Lacombe, AB (2011)	168.8±0.0	97.00±0.00	13.69±0.14	11.51±0.06
Melita, MB (2011)	200.0±8.8	97.00±1.41	13.24±2.31	10.94±0.32
Outlook, SK(2012)	193.8±0.0	95.00±1.41	12.55±0.12	10.93±0.03
Saskatoon-Sutherland, SK (2012)	181.3±0.0	96.00±0.00	12.68±0.46	10.82±0.27
g) Snowdrop				
Edmonton, AB (2011)	187.5±8.8	94.00±0.00	14.14±0.18	10.80±0.02
Saskatoon-Sutherland, SK (2011)	200.0±8.8	94.00±0.00	13.57±0.63	10.88±0.23
Rosthern, SK (2012)	181.3±0.0	94.50±0.71	14.26±1.29	10.65±0.39
Meath Park, SK (2012)	175.0±8.8	96.50±0.71	15.37±0.12	10.59±0.02

Values represent the mean±one standard deviation ($n=3$)

those of several commercial isolates, including those from soybean, pea, egg, whey, and wheat. Commercial production of isolates may result in varying protein functionality depending on the processing technique or conditions used. For instance, the use of spray drying in commercial production of protein isolates compared with freeze drying is reported to result in lower protein solubility (32). The relatively large standard deviations in some cases reflect variability because of the environment (year and/or location) across Western Canada for each genotype. The mean foaming capacity of faba bean protein isolates (162%) was found to be significantly higher than that of egg protein isolate (112%) ($p<0.01$) and similar to isolates from pea (150%) and soybean (157%) ($p>0.05$); however, it was lower than the foaming capacity of isolates from wheat (223%) and whey (228%) ($p<0.01$) (Table 4). In contrast, the mean foaming stability of faba bean isolates (65%) was found to be similar to all the commercial isolates (pea=56%, soy=58%, whey=69%, egg=73%,

and wheat=76%) ($p>0.05$).

The mean oil holding capacity of faba bean isolates (5.7 g/g) was found to be higher than all the commercial isolates (pea=1.1 g/g, egg=1.5 g/g, soy=1.6 g/g, whey=1.8 g/g, and wheat=2.3 g/g) ($p<0.01$). The mean solubility of the faba bean isolates (81%) was found to be higher than the isolates from wheat (11%), pea (20%), and soybean (31%) ($p<0.05$), but similar to the isolates from egg (88%) and whey (89%) ($p>0.05$). The mean emulsion capacity of faba bean isolates (184 g/g) was similar to the isolates from soybean (175 g/g), pea (181 g/g), and egg (184 g/g) ($p>0.05$), lower than the isolate from whey (206 g/g) ($p<0.01$), but higher than the isolate from wheat (94 g/g) ($p<0.01$). In contrast, the mean creaming stability of faba bean isolates (94%) was similar to the isolates from whey (89%) and egg (96%) ($p>0.05$), lower than the isolate from soybean (100%), but higher than the isolates from pea (50%) and wheat (62%) ($p<0.01$). The mean emulsification index of faba bean isolates (13.0 m²/g) was

Table 4. Foaming capacity, foam stability, emulsion capacity, creaming stability, oil holding capacity, emulsion activity and stability indices, and protein solubility for faba bean protein isolates and commercial protein isolates

Protein	Foaming capacity (%)	Foam stability (%)	Emulsion capacity (g/g)	Creaming stability (%)	Oil holding capacity (g/g)	Emulsion activity index (m ² /g)	Emulsion stability index (min)	Solubility (%)
Faba bean	162±5 ^a	65±2 ^{ab}	184±5 ^a	94±2 ^{ab}	5.7±0.2 ^a	13.0±0.7 ^{ab}	10.7±0.2 ^a	81.5±4.7 ^a
Soybean	157±5 ^a	57±5 ^a	175±9 ^a	100±0 ^c	1.6±0.3 ^{bc}	14.6±1.7 ^b	12.0±0.6 ^{ab}	30.5±0.1 ^b
Pea	150±5 ^a	55±1 ^a	181±0 ^a	50±0 ^d	1.1±0.0 ^b	1.7±0.0 ^c	11.9±0.2 ^{ab}	20.1±0.2 ^c
Whey	228±7 ^b	69±1 ^b	206±0 ^b	89±1 ^a	1.8±0.0 ^{cd}	14.5±0.4 ^b	13.0±0.7 ^b	89.0±1.4 ^d
Egg	112±2 ^c	73±4 ^b	188±9 ^a	96±0 ^{bc}	1.5±0.0 ^{bc}	11.5±0.1 ^a	11.6±0.1 ^{ab}	88.1±0.2 ^d
Wheat	223±5 ^b	76±1 ^b	94±0 ^c	62±3 ^e	2.3±0.0 ^d	1.2±0.2 ^c	13.1±0.2 ^b	10.7±0.7 ^e

Data for the faba bean protein isolates represent the mean value for all faba bean samples (7 genotypes x 2 locations x 2 years x triplicate measurements)±one standard deviation ($n=84$). Data for the commercial protein isolates represent the mean value of triplicate measurements±one standard deviation ($n=3$). Means in each column followed by different letters are significantly different ($p<0.05$)

found to be similar to the isolates from egg (11.5 m²/g), whey (14.5 m²/g), and soy (14.6 m²/g) ($p>0.05$), but higher than the EAls of isolates from wheat (1.2 m²/g) and pea (1.7 m²/g) ($p<0.01$). Similarly, the mean emulsification stability index of faba bean isolates (10.7 min) was found to be similar to that of isolates from egg (11.6 min), pea (11.9 min), and soybean (12.0 min) ($p>0.05$), but lower than the isolates from whey (13.0 min) and wheat (13.1 min) ($p<0.05$).

In summary, the effect of genotype on the composition and the physicochemical and functional properties of faba bean protein isolates were observed to be minimal. This is significant as secondary processors need not be concerned about the variety or growing location of their feedstock from an ingredient functionality standpoint. However, it should be noted that the levels of vicine and convicine in the isolate were not considered as part of this study. Because of concerns with respect to the association of vicine and convicine with favism, a toxicological evaluation of faba bean isolates should be performed prior to their use as food ingredients. The higher protein solubility of the faba bean isolates prepared in this study might be advantageous in protein drinks, infant formulas, and milk replacers, whereas higher oil holding capacities may play an important role in meat products by reducing fat loss and improving binding properties. Moreover, the emulsifying properties of faba bean isolates indicate their potential significance in the production of cake batters, bread, meat sausages, salad dressings, and dairy products.

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