THE EFFECT OF GENOTYPE AND THE ENVIRONMENT ON THE PHYSICOCHEMICAL AND FUNCTIONAL ATTRIBUTES OF FABA BEAN PROTEIN ISOLATES

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science in the

Department of Food and Bioproduct Sciences

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

By

Ashish Singhal

2015

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying, publication, or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of Department of Food and Bioproduct Sciences
University of Saskatchewan
Saskatoon, Saskatchewan, S7N 5A8
Canada

ABSTRACT

The overarching goal of this research was to investigate the differences in the physicochemical and functional properties of protein isolates produced from seven different faba bean genotypes (CDC Fatima, Taboar, SSNS-1, FB9-4, FB18-20, Snowbird and CDC Snowdrop) grown at different locations in Canada (Saskatchewan, Alberta and Manitoba) in 2011 and 2012. The protein isolates were prepared by alkaline extraction (pH 9.5) followed by isoelectric precipitation at pH 4.5. The isolates had an average protein content of ~94% and average protein and isolate yields of ~77% and ~25%, respectively. The physicochemical properties assessed in this study included surface charge/zeta potential (ZP), surface hydrophobicity (SH), and surface and interfacial tension (ST and IT). The functional properties tested included foaming capacity (FC) and foam stability (FS), emulsion capacity (EC) and creaming stability (CS), emulsion activity index (EAI) and emulsion stability index (ESI), oil holding capacity (OHC), and protein solubility.

The findings indicated that all physicochemical properties for all isolates were independent of genotype. Overall, an average ZP of + 22.1 mV, SH of 47.2 arbitrary units, and ST and IT of 65.0 mN/m and 10.7 mN/m, respectively, were observed. However, with the exception of ZP considerable differences were observed due to the effect of environment. The ratio of the major globulin protein fractions [legumin:vicilin (L/V)] was found to shift during processing, from 3.8 (range: 3.4-4.6) in the flour to 4.5 (range 4.0-4.9) in the isolates. The L/V ratio for faba bean flour and isolate samples was also found to be independent of genotype. For all genotypes, with the exception of the zero-tannin varieties (Snowbird and CDC Snowdrop), the L/V ratio was affected by the environment.

Similar to the physicochemical properties, all functional attributes were found to be independent of genotype. However, environmental effects were observed for all functional properties with the exception of EAI and ESI. Average values for FC of 162.0%, for FS of 65.0%, for EC of 184.0 g/g, for CS of 94.0%, for OHC of 5.7 g/g, for EAI of 13.0 m^2/g, for ESI of 10.7 min and for solubility of 81.0% were reported. Zeta potential was observed to be positively correlated with CS ($r = 0.46$; $p<0.05$) and FS ($r = 0.54$; $p<0.01$), whereas SH and L/V ratio were not. The L/V ratio in the isolate, however, was correlated positively with SH ($r = 0.40$; $p<0.05$) and negatively with ZP ($r = -0.39$; $p<0.05$). Moreover, the solubility of faba bean

isolates was found to be positively correlated with ZP ($r = 0.44$; $p<0.05$) and negatively correlated with both IT ($r = -0.38$; $p<0.05$) and OHC ($r = -0.38$; $p<0.05$).

The functional properties of some commercial protein isolates (soy, pea, whey, egg and wheat) were evaluated for comparative purposes. The OHC of the faba bean isolate was found to be higher than that of any of the commercial isolates. With the exception of CS (soy and pea) and FC (egg), all of the emulsifying (EC, EAI and ESI) and foaming (FC and FS) properties of the faba bean protein isolates were comparable to those of soy, pea and egg isolates. In contrast, values for most of the other functional properties were greater for faba bean isolates than for the pea and wheat isolates, but lower than for the whey isolate. For example, the solubility of the protein isolates was observed to decrease in the following order: whey (89.0%) = egg (88.1%) > faba bean (81.0%) > soybean (30.5%) > pea (20.1%) > wheat (10.7%).

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude for all of the help, support and incredible reinforcement put forth by my supervisors, Drs. Michael Nickerson and Robert Tyler. I would also like to thank my Advisory Committee members, Drs. Mehmet Tulbek (AGT Food and Ingredients, Saskatoon, SK) and Albert Vandenberg (Department of Plant Sciences, University of Saskatchewan), and my graduate chair, Dr. Xiao Qui, for providing excellent advice and constructive criticism to enable me to progress through my project. I would also like to thank my external examiner, Dr. Tom Scott from the Department of Animal and Poultry Science for his time and careful review of my thesis. I greatly appreciated and would like to acknowledge POS Bio-Sciences Corp. (Saskatoon, SK) and Crop Development Centre (Saskatoon, SK) staff for their technical support. I would like to thank Dr. Shahram Emami, Heather Silcox and Yuanlong Cao for their technical training and for providing technical support from time to time. I am also grateful to Dr. Eric Lamb for providing advice on statistical analysis of my data. I am also very grateful for the assistance and friendship from my colleagues Dr. Aleksandar Yovchev, Chang Chang, Andrea Stone, Dr. Angelika Teymurova, Lam Lam Cheung, Dr. Jiapei Wang, Angie Lam, Erin Hopkins, Nicole Avramenko, Dr. Ricky Lam, Ayanti Matharage, Yun Wang, Tian Bai, Yun Wang, Gebreegziabher Gebremedhin, Maria Martinez, Jamie Willems and Natalia Varankovich. I would also like to thank Ann Harley, Patricia Olesiuk, Tanya Napper and Kendra Panko for their administrative support. Thank you to all my family and friends for helping me maintain my sanity through the process and a very special thanks to my loving and supportive parents Mr. Raj Kumar Singhal and Mrs. Poonam Singhal for being by my side through this enriching journey. I would also like to thank Vivek Vardhan Reddy, Suneru Perera, Jenifer Siril and Oarabile Kgosisejo for their continuous support during this journey. Lastly, I am thankful for the financial support which was provided by the Saskatchewan Agriculture Development Fund, the Western Grains Research Foundation, and Saskatchewan Pulse Growers.

TABLE OF CONTENTS

Page

PERMISSION TO USE ..... **i**

ABSTRACT ..... **ii**

ACKNOWLEDGEMENTS ..... **iv**

TABLE OF CONTENTS ..... **v**

LIST OF TABLES ..... **viii**

LIST OF FIGURES ..... **x**

LIST OF SYMBOLS AND ABBREVIATIONS ..... **xii**

1. INTRODUCTION **1**

1.1 Overview 1

1.2 Objectives 3

1.3 Hypotheses 3

2. LITERATURE REVIEW **4**

2.1 Pulse proteins: An overview 4

2.2 Protein structure and legumin/vicilin (L/V) ratio 5

2.3 Protein extraction 8

2.3.1 Dry processing 8

2.3.2 Wet processing 9

2.4 Functional properties of legume proteins 13

2.5 Faba bean (*Vicia faba* L.) 20

3. MATERIALS AND METHODS **24**

3.1 Materials 24

3.2 Processing of faba bean seed 27

3.3 Preparation of faba bean protein isolates 27
3.4 Proximate analysis 28
3.5 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis 28
3.6 Colour 29
3.7 Physicochemical properties 29
3.7.1 Surface charge (zeta potential) 29
3.7.2 Intrinsic fluorescence 30
3.7.3 Interfacial tension 30
3.8 Functional properties 31
3.8.1 Oil holding capacity 31
3.8.2 Foaming capacity and stability 31
3.8.3 Emulsion capacity 32
3.8.4 Creaming stability 32
3.8.5 Emulsion activity and stability indices 32
3.8.6 Protein solubility 33
3.9 Statistics 34

4. RESULTS 35
4.1 Proximate analysis 35
4.2 Extraction efficiency of isolate production 40
4.3 Protein composition of flours and isolates 40
4.4 Colour of flours and isolates 44
4.5 Physicochemical properties of the protein isolates 46
4.5.1 Zeta potential 46
4.5.2 Surface hydrophobicity 48
4.5.3 Surface and Interfacial tension 48
4.6 Functional properties of the protein isolates 52
4.6.1 Oil holding capacity 52
4.6.2 Emulsion capacity and creaming stability 52
4.6.3 Emulsion activity and stability indices 54
4.6.4 Foaming capacity and stability 58

4.6.5 Protein solubility 60
4.7 G x E relationships 62
4.8 Structure-function relationships 63
4.9 Principal Component Analysis (PCA): Scatter Plot 65
4.10 Comparison to commercial isolates 68

5. DISCUSSION 70
5.1 Colour characteristics and proximate composition of faba bean flours and isolates 72
5.2 Extraction efficiencies of isolate production 73
5.3 Protein composition of flours and isolates 73
5.4 Functional properties of isolates and comparison with commercial isolates 75
5.4.1 Oil holding capacity 76
5.4.2 Emulsifying capacity, creaming stability, emulsifying activity and stability indices 77
5.4.3 Foaming capacity and stability 79
5.4.4 Protein solubility 80
5.5 G x E analysis 81
5.6 Applications 82
5.7 Factors affecting growth of faba bean and chemical composition 84

6. CONCLUSIONS AND FUTURE STUDIES 86

7. REFERENCES CITED 89

LIST OF TABLES

Page

Table 2.1 Approximate chemical composition of faba bean flour, protein isolates, and alkaline residue. All measurements are in % (w/w) 21

Table 3.1 Seven faba bean genotypes grown at several locations within Saskatchewan, Alberta and Manitoba in 2011 and 2012.....26

Table 3.2 Average monthly temperature and precipitation data for April to October over a two year duration (2011–2012) at locations where faba bean seed was grown within Saskatchewan, Alberta and Manitoba.....27

Table 4.1 Percent protein, crude fat and ash in faba bean flours produced from seven genotypes in two years at several locations in Western Canada. Data represent the mean ± one standard deviation (n = 2).....36

Table 4.2 Percent protein and ash in faba bean protein isolates produced from seven genotypes in two years at several locations in western Canada. Data represent the mean ± one standard deviation (n = 2) 39

Table 4.3A L*, a* and b* values of faba bean flours prepared from seven genotypes grown in 2011 and 2012 at several locations in Western Canada. Values represent the mean ± one standard deviation (n = 3). 45

Table 4.3B L*, a* and b* values of faba bean protein isolates prepared from seven genotypes grown in 2011 and 2012 at several locations in western Canada. Values represent the mean ± one standard deviation (n = 3). 47

Table 4.4 Pearson correlation coefficients (r) for physicochemical, colour, seed size, functional, proximate, and L/V properties of faba bean flour and protein isolates from all genotypes. 64

Table 4.5 Pearson correlation coefficients (r) for physicochemical, colour, seed size, functional, proximate, and L/V properties of faba bean flour and protein isolates from SSNS-1, FB9-4 and FB18-20 genotypes.....66

Table 4.6 Foaming capacity (FC) and stability (FS), emulsion capacity (EC), creaming stability (CS), oil holding capacity (OHC), emulsion activity (EAI) and stability (ESI) indices, and protein solubility (Sol) for faba bean protein isolates as a function of genotype. Data for the faba bean protein isolates represents the mean value for each genotype (2 locations x 2 years x duplicate measurements) ± one standard deviation (n = 8). Data for the commercial protein isolates represents the mean value of duplicate measurements ± one standard deviation (n = 2)... .69

LIST OF FIGURES

Page

Figure 2.1 Relative protein precipitation ability of cationic and anionic salts 12

Figure 3.1 Images of faba bean genotypes with (A) small, (B) intermediate (C) and largest seed sizes 25

Figure 3.2 Major soil zones and locations in the prairie region 26

Figure 4.1 Representative examples of: A) SDS-PAGE gel for isolate (Ai) and flour (Aii) prepared from FB18-20 grown at Edmonton in 2011; and (B) a corresponding densitometry analysis for the isolate protein profile 41

Figure 4.2 L/V ratios of faba bean isolates (A) and faba bean flours (B) as a function of genotype, location and year at pH 7.0. Data represent means ± one standard deviations (n=2). 43

Figure 4.3 Surface charge (or zeta potential) (A) and intrinsic florescence (B) of faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n = 2). 49

Figure 4.4 Surface (A) and interfacial (B) tension of faba bean protein isolates at the air-water and oil-water interface, respectively, as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n = 2) 51

Figure 4.5 Oil holding capacity (g/g) of faba bean protein isolates as a function of genotype, location and year. Data represent the mean ± one standard deviation (n = 2). 53

Figure 4.6 Emulsion capacity of faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n = 2).....55

Figure 4.7 Creaming stability (%) of faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n=2).....56

Figure 4.8 Emulsion activity (A) and stability (B) indices for faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n=2).....57

Figure 4.9 Foaming capacity (A) and stability (B) of faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n=2).....59

Figure 4.10 Protein solubility (%) of faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n=2).....61

Figure 4.11 The Scatter plot of the first two principal components (p1 and p2), depicting the distribution of all faba bean genotypes, locations and years in the PCA model. 68

LIST OF SYMBOLS AND ABBREVIATIONS

a*	Red-green colour, with red associated with higher values of a*
A_0	Absorbance of the diluted emulsion
ΔA	Absorbance difference
a.u.	Arbitrary units
AB	Alberta
ANFs	Anti-nutrient factors
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
b*	Yellow-blue colour, with yellow associated with higher values of b*
c	Concentration of protein solution
°C	Degree Celsius
CS	Creaming stability
d.b.	dry basis
DF	Diafiltration
DH	Degree of hydrolysis
EAI	Emulsion activity index
EC	Emulsion capacity
ESI	Emulsion stability index
FC	Foaming capacity
FI	Fluorescence intensity
f (kα)	Smoluchowski approximation
F_{max}	Maximum force
FS	Foam stability
g oil/ g protein	Grams oil per gram protein
G x E	Genotype x environment
G x L	Genotype x location
G x Y	Genotype x year
G6PD	Glucose-6-Phosphate Dehydrogenase
ICARDA	International Center for Agricultural Research in the Dry Areas

IgE	Immunoglobulin E
IEP	Isoelectric precipitation
IT	Interfacial tension
κ	Debye length
kDa	Kilodalton
L*	Brightness, 0 to 100, moving from dark to light
L_s, a_s and b_s	Standards for calibration of Hunter lab colorimeter
L/V	Legumin to vicilin ratio
M	Molar
MB	Manitoba
mM	Millimolar
mN/m	Milli-Newton per metre
mV	Millivolt
η	Viscosity
n	Dilution factor
N	Normality
%N	%Nitrogen
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
NFDM	Non-fat dry milk
O/W	Oil-in-water
OHC	Oil holding capacity
p1 and p2	Principal components
PCA	Principal component analysis
pI	Isoelectric point
PMM	Protein micellar mass
PSE	Protein separation efficiency
R	Radius of the ring
RP-HPLC	Reverse phase high performance liquid chromatography
rpm	Revolution per minute
S	Svedberg Unit
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SH	Surface hydrophobicity
SK	Saskatchewan
Sol	Solubility
ST	Surface tension
TIA	Trypsin inhibitor activity
U_E	Electrophoretic mobility
UF	Ultrafiltration
V	Volt
v/v	Volume to volume
V_A	Volume of the aqueous phase after homogenization
V_B	Volume of the aqueous phase before homogenization
V_{F0}	Volume of the foam at time 0 min
V_{F30}	Foam volume after 30 min
V_{sample}	Initial volume of sample used
W/O	Water-in-oil
w/v	Weight to volume
w/w	Weight to weight
WHC	Water holding capacity
Wt_{Dry}	Weight of dry sample
Wt_{Wet}	Weight of wet sample
ZP	Zeta potential
α	Radius of the particle
β	Correction factor
ε	Permittivity
ζ	Zeta potential
φ	Oil volume fraction
γ	Interfacial tension

1. INTRODUCTION

1.1 Overview

In human food, proteins from different sources are consumed for their nutritional and health promoting benefits. However, in recent times, food industries are leaning towards plant-based alternatives in the formulation of food products due to "increasing dietary preferences among consumers; growing cost difference between animal and plant proteins; and rising concerns over the safe consumption of animal-based products" (Liu et al., 2010). 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 (Ustimenko-Bakumovsky, 1983; Schutyser et al., 2015). Pulses can be used in many ways, either through the direct consumption of regional crops (a frequent practice in developing countries) including chickpea (*Cicer arietinum*), pea (*Pisum sativum*) and faba bean (*Vicia faba* L.), or as functional ingredients (flours, concentrates, 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 (Vioque et al., 2012) and has a high protein level (32.5% on a dry weight basis, Gueguen and Cerletti, 1994). It has a well-balanced amino acid composition when consumed with cereals (Alamanou et al., 1996; Dervas et al., 1999; Pozani et al., 2002) and is a good source of vitamins, minerals and fibre (Ofuya and Akhidue, 2005). However, its use in the form of a protein isolate is very limited, in part because of the poor understanding of structure-function relationships, a decrease in production of faba bean (Rubiales, 2011), and the presence of anti-nutritional compounds such as glycosides which are reported to cause favism, a severe form of anemia in some people (McMillan et al., 2001).

The major storage proteins in pulses, known as globulins, are categorized into legumins (L) (11S, S = Svedberg unit), vicilins (V) (7S) and convicilin. The legumin:vicilin (L/V) ratio is not fixed and varies among cultivars (Gatehouse et al., 1980; Martensson, 1980; Casey et al., 1982; Schroeder, 1982; Murphy and Resurrecceon, 1984; Gueguen and Barbort, 1988; Kim et al., 1994; Cai and Chang, 1999; Mujoo et al., 2003; Tzitzikas et al., 2006; Mertens et al., 2011). 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 colour, seed colour and size (Black et al., 1998; Vidal-Valverde et al., 2003) whereas extrinsic factors comprise of agronomic factors, and method of analysis (Mertens et al., 2011). Variation in the L/V ratio may influence the physicochemical and functional properties of pulse protein products (Swanson, 1990; Utsumi et al., 1997; Can Karaca et al., 2011). For instance, Bora et al. (1994) observed that 7S pea globulins underwent heat gelation, whereas 11S globulins did not under the same processing conditions. Barac et al. (2010) reported that pea genotypes with high 7S protein levels or low 11S protein levels yielded higher amounts of protein (higher protein extractability) as compared to other genotypes. The Mertens et al. (2011) study on smooth pea reported significant differences in protein content and L/V ratio for different genotypes, locations and years of growth. The variability in the L/V ratio could also be used in preparation of a wide range of foods with specific functional attributes such as foaming, emulsification or gelation. However, limited work has been done which describes the impact of environmental factors on the functionality of faba bean protein isolates, and the relationship between the L/V ratio and its functional attributes.

The overall goal of this research was to investigate structure-function relationships determining the functionality of faba bean protein isolates in response to genotype and growth environment. Seven genotypes (CDC Fatima, Taboar, SSNS-1, FB9-4, FB18-20, Snowbird and CDC Snowdrop) grown in 2011 and 2012 at various locations in Canada (Manitoba, Saskatchewan and Alberta) were assessed. These genotypes were selected based on their physical and chemical characteristics such as tannin level, seed size and colour. For instance, both Snowbird and CDC Snowdrop are zero tannin genotypes for which seeds are observed to be very light in colour with no redness as compared to other tannin containing genotypes which are darker in colour with a redness character. Although the effect of environment was examined, a true genotype X environment study could not be undertaken due to limited seed availability from some locations. A genotype x location and genotype x year effect was studied only for SSNS-1, FB9-4 and FB18-20 which were grown at same locations in 2011 and 2012. It was hypothesized that the L/V ratio would be a significant determinant of the functionality (e.g., solubility, foaming, emulsification, oil/water holding, etc.) of faba bean protein isolates, and that functionality would differ depending on the genotype and the environmental conditions in which the seed was grown. Confirmation of these hypotheses might make possible the selection of

genotypes or production lots particularly suitable for producing isolates to compete in the plant protein market.

1.2 Objectives

The specific objectives of this research were:

1) To characterize the effect of genotype and environment on the composition, including the legumin:vicilin (L/V) ratio, of faba bean flours and protein isolates;
2) To characterize the effect of genotype and environment on the physicochemical properties of faba bean protein isolates;
3) To characterize the effect of genotype and environment on the functional properties of faba bean protein isolates, and to compare them to those of commercial protein ingredients; and
4) To discern structure-function relationships.

1.3 Hypotheses

The specific hypotheses of this research were:

1) Significant differences exist in the protein composition and the legumin:vicilin (L/V) ratio of faba bean flours and protein isolates and these differences are related to genotype and environment;
2) Significant differences exist in the functional and physicochemical properties of faba bean protein isolates and these differences are related to genotype and environment;
3) Significant correlations exist between the physicochemical and functional properties and the L/V ratio of faba bean protein isolates.

2. LITERATURE REVIEW

2.1 Pulse proteins: An overview

Pulses such as beans, peas and lentils have been consumed for thousands of years and represent one of the most extensively consumed food in the world (Mudryj et al., 2014). Pulses play crucial roles in fulfilling the nutritional requirements of the growing population in a cost effective manner, especially for developing or underdeveloped countries where animal protein consumption is either limited or expensive (Aguilera et al., 2013). Pulses are widely used for food purposes because of their high protein content; high nutritional and health beneficial properties; appropriate functional attributes; and associated low production cost and abundance (Duranti, 2006). The health benefits associated with pulse consumption include lowering of cholesterol levels, reducing the risks of various cardiovascular diseases and cancers, and decreasing the risk of type-2 diabetes (Roy et al., 2010). Along with protein, pulse provides dietary fibre and, vitamins and minerals such as iron, zinc, folate, and magnesium (Mudryj et al., 2014). Pulses also have an antioxidant and anti-carcinogenic effect because of the presence of phytochemicals, saponins and tannins in them (Mudryj et al., 2014).

For many years, pulses have been used in the preparation of wholesome nutritional meals in combination with other food sources or ingredients. Pulse crops such as pea, chickpea and common bean (*Phaseolus vulgaris* L.), when blended with regionally grown cereal grains, could be of immense value in helping to fulfill the nutritional requirements of people relying just on mono-carbohydrate diets (Boye et al., 2010a). However, the nutritional quality of pulses is limited because of the presence of heat labile and heat stable anti-nutritional factors (ANFs) (Aguilera et al., 2013). The ANFs include proteins such as lectins and protease inhibitors, and other compounds such as phytate, tannins, saponins, and alkaloids (Aguilera et al., 2013). The negative impact of these ANFs on consumption of pulses in human and animal diets has been extensively reported (Adebowale et al., 2005). However, the processed forms of legumes (flours, concentrates or isolates) reported to have lower levels of ANFs than their corresponding raw material form (seeds) (Hajos and Osagie, 2004). For instance, during the germination process,

legumes found to have a higher digestibility, soluble protein (Vernaza et al., 2012) and dietary fibre (Martin-Cabrejas et al., 2008; Benitez et al., 2013), and reduced levels of ANFs (Vidal-Valverde et al., 2002). Furthermore, protein isolates prepared by extraction or precipitation methods also found to have reduced anti-nutritional factors such as trypsin inhibitors, glycosides (such as convicine and vicine) and haemagglutinins which would otherwise impair protein digestion and could be toxic for human consumption (Frias et al., 1995; Kothekar et al., 1996; Kozlowska et al., 1996; Boye et al., 2010a). The exploitation of protein isolates or concentrates in new food formulations is of great importance because of their high nutrition and functionality (Lecomte et al., 1993). The utilisation of right individual functional properties might be useful in producing different food products such as cakes, biscuits, beverages and breads.

2.2 Protein structure and legumin/vicilin (L/V) ratio

The majority of pulse proteins are albumin and globulin fractions, where globulins represent ~70% and albumins constitute 10-20% of the total pulse protein (Boye et al., 2010a; Can Karaca et al., 2011). In addition, other proteins which are present in minor proportions such as prolamins and glutelins (Gupta and Dhillon, 1993; Saharan and Khetarpaul, 1994). These four proteins can be classified according to their solubility in various solvents based on the Osborne classification scheme (Osborne, 1924). For example, globulin proteins are soluble in dilute salt solution, albumins in water, prolamins in 70% ethanol solution, and glutelins are solubilized in dilute alkali solutions (Osborne, 1924; Oomah et al., 2011).

Albumins encompass structural and enzymatic proteins, lectins and protease inhibitors, with their overall molecular mass (MM) ranging between 5 and 80 kDa (Boye et al., 2010a). In contrast, the salt soluble globulins include legumin (11S, S = Svedberg Unit) and vicilin (7S) proteins. The 11S fraction is a hexamer (MM of ~340-360 kDa) comprised of six subunits (MM of ~60 kDa) linked by non-covalent interactions. Each subunit pair is comprised of an acidic (MM of ~40 kDa) and basic (MM of ~20 kDa) chain joined by a disulfide bond (Swanson, 1990; Can Karaca et al., 2011). In contrast, the 7S fraction is a trimer with a MM of ~175-180 kDa, and lacks disulfide bridging (Boye et al., 2010a). Vicilin protein molecules also have been reported to have various subunits of 75, 43, 33, 56, 12 and 25 kDa (Swanson, 1990; Can Karaca et al., 2011). A third type of globulin is also present, although in lesser amounts as compared to other globulins, and is known as convicilin (Croy et al., 1980). It is a 7S globulin, and a single

convicilin molecule has an overall MM of 220-290 kDa, and consists of 3 or 4 subunits each with a MW of 70 kDa. This protein has a different amino acid profile than vicilin as it contains sulfur-containing amino acids, is immunologically similar to 7S vicilin, and contains very little carbohydrate (Boye et al., 2010a). Various pulse species have been reported to contain convicilin-type proteins. For example, Saenz de Miera et al. (2008) investigated 29 different legume species from 4 genera (*Pisum, Lens, Vicia* and *Latyruus spp.*), and reported the presence of 34 new convicilin gene sequences. All of the above studies considered convicilin as a third class of globulin molecules. However, O'Kane et al. (2004) deny the consideration of convicilin as a third pea globulin based on their findings and reported that convicilin (a polypeptide) should be denoted as the R-subunit of pea vicilin molecules (salt extracted).

The ratio of legumin:vicilin (L/V) is not fixed and may vary among different pulse varieties and species. The ratio of L/V for pea, soybean and faba bean varies in the range of 0.2-8.0, 1.3-3.4 and 1.7-3.7, respectively (Gatehouse et al., 1980; Martensson, 1980; Casey et al., 1982; Schroeder, 1982; Murphy and Resurrecceon, 1984; Gueguen and Barbort, 1988; Kim et al., 1994; Cai and Chang, 1999; Mujoo et al., 2003; Tzitzikas et al., 2006; Mertens et al., 2011). Various studies reported that L/V ratio for wrinkled pea seeds (0.2-0.6) represent a smaller ratio compared to the smooth pea seeds (0.3-2.0) (Schroeder, 1982; Gueguen and Barbot, 1988; Cousin, 1997; Mertens et al., 2011). Various factors including the methods used in the preparation of protein materials (concentrates or isolates); processing parameters like pH and temperature and environmental or agronomic factors may account for the variation in these ratios, which in turn could also have influential effects on the physiochemical properties of pulse protein materials (Swanson, 1990; Utsumi et al., 1997; Can Karaca et al., 2011). As a part of their studies, Barac et al. (2010) extracted the proteins from six varieties (genotypes) of pea (Calvedon, L1, L2, L3, Maja and M.A) and indicated that genotypes with high 7S protein levels or low 11S protein levels yielded higher amounts of protein (protein extractability) compared to the other genotypes. Moreover, pure vicilin solutions were observed to have better functional properties (such as emulsification and gelation) than the pure legumin solutions (Barac et al., 2010). It was indicated that a low L/V ratio for preparation of protein isolates could be desirable. In the Mertens et al. (2011) study on smooth pea seeds, it was reported that agronomic factors, including variety, cultivar type and location, affected the protein content and L/V ratio with high significance. However, some varieties were less sensitive to the prevailing climatic conditions

than others. This approach could be beneficial from an industrial point of view as it could manifest in picking stable and less sensitive L/V ratio lines for specific product quality characteristics (Mertens et al. 2011).

Various groups have researched relationships between L/V ratios and their functional attributes. A number of studies noted that pea vicilin showed higher emulsifying properties than corresponding pea legumin (Cserhalmi et al., 1998; Rangel et al., 2003; Kimura et al., 2008), which was attributed due to higher solubility (Koyoro and Powers, 1987) and surface hydrophobicity (Boye et al., 2010a) of vicilin proteins. Furthermore, Shen and Tang (2014) reported that emulsifying properties of vicilins were found to be dependent on both the legume source (Kidney bean, red bean and mung bean) and their protein concentration (0.25-2.5% w/v). The differences in the emulsion properties of vicilins at different concentrations were majorly related to the variation in zeta potential and interfacial characteristics, and were also found to be dependent on other factors such as protein folding, penetration and structural rearrangement at the interface (Shen and Tang, 2014). Bora et al. (1994) studied the heat induced gelation of mixed pea globulins and found that 7S globulin had the capacity to undergo heat gelation while 11S globulin did not although used the same optimal conditions of gelation with 15% globulin solutions, pH 7.1 and heating at 87°C for 20 min. However, Nakamura et al. (1986) observed that the gels formed by 7S globulins of soybean are less strong and transparent as compared to those formed by 11S globulins, which were much harder and turbid in nature. The study suggested that the extent of interaction in gel formation of a mixed system of 7S and 11S globulins is affected by factors such as the 11S/7S ratio and the composition of their subunits. Cserhalmi et al. (1998) reported that mixed globulins and 7S fractions of pea proteins had increased surface hydrophobicity and emulsifying properties compared to the albumins and 11S fractions. Moreover, for all the pea varieties tested, the emulsifying and surface hydrophobicity properties were different from each other. Thus, varying the L/V ratio could be used in obtaining the desired functional attribute in new food formulations.

The quantification of 7S and 11S fractions present in isolates or concentrates is an essential step for calculation of L/V ratio which can be achieved using various methods described in literature. Methods include ammonium sulfate salt extraction (Danielsson, 1949), isoelectric precipitation (Derbyshire et al., 1976), sodium dodecyl sulfate-polyacrylamide-gel electrophoresis (SDS-PAGE), gel chromatography (Gwiazda et al., 1980), selective thermal

denaturation (Varfolomeeva et al., 1985), sucrose gradient centrifugation (Mori and Utsumi, 1978) and zonal isoelectric precipitation (Scholz et al., 1974; Wright and Boulter, 1974). The effective separation and the choice of technique should be depend on factors such as nature of sample (isolates, concentrates, seed), extraction technique employed and the level of purification required. For testing of functional and physicochemical properties of 7S and 11S fractions, it is required that enough quantity of these samples is obtained whichever technique is used without compromising the purity.

2.3 Protein extraction

Protein extraction is dependent on many factors such as pH, temperature, particle size, ionic strength, type of salt used, and solvent to flour ratio (Kinsella, 1979; Aguilera and Gracia, 1989). Various extraction methods are being studied so to maximize the protein yield without compromising the protein functionality of the concentrate or isolate product. The protein extraction processes which are being exploited in the preparation of protein-rich materials (such as isolates and concentrates) can be classified into dry and wet methods (Tyler et al., 1981; Zheng et al., 1998; Tian et al., 1999).

2.3.1 Dry processing

Dry processing of pulses is typically done by air classification, which involves the separation of flours on the basis of particle size and density using an air stream into protein and starch rich fractions (Tyler, 1984; Swanson, 1990). Air classification has been found to be suitable for legume crops low in fat, such as field pea and common bean. Flours are first fractionated into starch (SI) and protein (PI) rich concentrates using an air classification method. SI is then remilled and fractionated to give SII and PII concentrates (Tyler et al., 1981). Protein separation efficiency (PSE) is defined as the percentage of total flour protein recovered in the PI and PII fractions, and measured as the subtraction of % total flour protein recovered in SII fraction from 100% (Tyler et al., 1981). For legume crops high in fat such as soybean and chickpea, particle agglomeration is detected which interferes with PSE (Sosulski and Youngs, 1979; Gueguen, 1983; Gueguen and Cerleti, 1994). Dry processing has major advantage over wet extraction methods as the native functionality of proteins is retained and a lower amount of energy and water is required (Pelgrom et al., 2013). Moreover, in contrast to wet extraction

methods where both protein concentrates and isolates can be produced, dry processes are suitable only for preparing protein concentrates with protein content from 40 to 75% (Kiosseoglou and Paraskevopoulou, 2011) probably because of the presence of higher amount of other compounds such as oil and fibres, and protein loss in coarse fractions (Schutyser et al., 2015).

Tyler et al. (1981) studied the fractionation of eight legumes (cowpea, great northern bean, lima bean, mung bean, navy bean, lentil, faba bean and field pea) using flours produced by pin milling followed by air classification and found faba bean (63.8-75.1%) and lima bean (43.4-49.6%) to have the highest and lowest protein concentrations in the protein-rich fractions. According to the authors, the suitability of pin milling followed by air classification is strongly correlated with the PSE of the legumes. Mung bean, lentil and great northern bean were found to have the highest mean PSE values of 88.9, 87.2 and 87.0%, respectively, whereas lima bean, cowpea and navy bean showed the lowest at 80.2, 78.2 and 80.3%, respectively. The other two legumes, faba bean and field pea, had PSE values of 84.1 and 82.8%, respectively. Overall, the authors indicated that except for lima bean and cowpea, the legumes were found to be suitable for separation of protein and starch fractions by the pin milling and air classification method.

2.3.2 Wet processing

In general, wet extraction methods can be exploited for preparing both protein concentrates and isolates at levels of 70% and 90% protein (or higher), respectively. However, it should be noted that currently there is no universal classification scheme which separates concentrate from an isolate for all the legumes. The various wet extraction processes include acid/alkaline extraction-isoelectric precipitation, ultrafiltration and salt extraction. Legume flours dispersed in aqueous solutions typically show high solubility when subjected to alkaline or acidic extraction conditions at pH 8-10 and below 4 respectively (Kiosseoglou and Paraskevopoulou, 2011).

a) Acid/alkaline extraction-isoelectric precipitation (IEP):

Briefly, proteins are first dissolved under alkaline (alkaline extraction) or acidic (acid extraction) conditions, followed by a clarification step and then precipitation by adjusting the pH to the isoelectric point (pI) of the protein (Han and Hamaker, 2002). In solutions with the pH < pI, proteins assume a net positive charge, whereas at pHs > pI proteins assume a net negative

charge. Under solvent conditions where proteins carry a net positive or negative charge, repulsive forces between proteins repel neighbouring molecules, and also promote protein-water interactions for improved dispersion and solubility. Near the pI value, proteins tend to carry a neutral net charge, allowing neighbouring proteins to aggregate via attractive van der Waals forces and hydrophobic interactions. Under these conditions, protein-protein interactions are favoured over protein-water interactions, and thus protein is precipitated out of the solution.

According to Han and Hamaker (2002), alkaline extraction followed by isoelectric precipitation is the most widely used method for obtaining extracts with protein purity greater than 70%. During alkaline extraction, legume proteins become solubilized at high pH values. The solution can then be clarified by centrifugation to remove insoluble material such as insoluble fibre, carbohydrates and insoluble proteins (e.g., prolamins). Protein concentrates or isolates can be formed by reducing the pH of the supernatant to near the pI of the protein using an acid such as HCl, (Boye et al., 2010b; Kiosseoglou and Paraskevopoulou, 2011). The study of Can Karaca et al. (2011) showed that isolates prepared from legumes (faba bean, chickpea, lentil, pea and soybean) by an alkaline extraction/IEP method had higher overall protein content (85.6%) as compared to those prepared by a salt extraction method (78.4%). Moreover, it was reported that both legume source and protein extraction method along with their interaction had significant effects on protein levels of isolates, and also on physicochemical and emulsifying properties. The overall surface charge, solubility, hydrophobicity and creaming stability for IEP produced isolates was higher as compared to isolates produced by salt extraction method (Can Karaca et al., 2011). The effect of processing or extraction conditions on the protein content of isolates can also be well observed from the studies of Flink and Christiansen (1973) and McCurdy and Knipfel (1990). In the former study, faba bean isolates with protein contents of 80.0-90.0% were obtained when the bean:solvent ratio was 1:5 (w/v) with pH 8 to 10 at 23°C for 10 min, and the precipitation of protein was carried out at pH 3-5. While in the latter study, the protein content of faba bean isolates was 76.4-94.0% using a bean:solvent ratio of 1:5 w/v with pH 7 to 10, 30 min, temperatures of 10°C and 20°C, and precipitation at pH 4-5.3.

Acid extraction (in principle similar to alkaline extraction) involves the preliminary extraction of proteins under acidic conditions. This process could result in high solubilisation of proteins prior to protein recovery (IEP, Ultrafiltration (UF)), as proteins tend to be more soluble under acidic conditions (pH below 4) (Boye et al., 2010a). In a study by Vose (1980) for

preparation of faba bean (*Vicia faba equina* L. cv. Diana) and pea (*Pisum sativum* L. cv. Trapper) IEP isolates, the cyclone discharge obtained from pin milling of these two legumes was acidified directly using 2 N HCl to a isoelectric point of 4.4-4.6. This process resulted in pea and faba bean protein isolates with 91.9% and 91.2% protein levels, respectively (Boye et al., 2010a).

b) Ultrafiltration/diafiltration

In the literature, membrane separation methods were shown to produce protein isolates with higher functionality (Fredrikson et al., 2001; Fuhrmeister and Meuser, 2003) and were effective in reducing levels of anti-nutritional components which include protease and amylase inhibitors, lectins and polyphenols (Singh, 1988; Waggle et al, 1989; Mondor et al., 2009). Ultrafiltration (UF) and microfiltration are membrane-based fractionation methods using pressure as the driving force for separation. Microfiltration can be used to separate particles or macromolecules larger than 0.1 µm, whereas ultrafiltration removes similar particles in the range of 0.001 to 0.02 µm (Koros et al., 1996). For preparation of protein materials using ultrafiltration, the supernatant after alkaline or acidic extraction is processed using either UF or UF and diafiltration (DF) together to isolate the protein material. Ultrafiltration is often combined with DF to improve protein recovery, where water is added to the retentate for dilution purposes, followed by re-ultrafiltration.

Vose (1980) used the UF procedure to produce faba bean and pea protein isolates which produced protein levels of 94.1% and 89.5%, respectively. Boye et al. (2010b) evaluated the protein content of isolates obtained from different pulses (pea, chickpea and lentil) using alkaline extraction-isoelectric precipitation (IEP) and UF/DF extraction methods. The protein content in concentrates obtained by the UF/DF method was found to be higher than in those obtained by IEP. For instance, for yellow pea, green lentil, red lentil, and desi and kabuli chickpea, UF/DF gave protein levels of 83.9%, 88.6%, 82.7%, 76.5% and 68.5%, respectively. In contrast, for IEP extraction, protein levels were 81.7%, 79.1%, 78.2%, 73.6% and 63.9% respectively for the same legume crops. Moreover, it was reported that UF was different from IEP in terms of protein composition as the isolates prepared by UF comprised both globulins and albumins, whereas the isolates prepared by IEP were observed to contain only globulins (Papalamprou et al., 2009; Papalamprou et al., 2010; Kiosseoglou and Paraskevopoulou, 2011).

c) Salt extraction

Salt extraction is a process where globulin proteins are separated from albumins on the basis of solubility (Boye et al., 2010a), as described previously in the Osborne classification scheme (Osborne, 1924). Proteins contain both hydrophobic and hydrophilic amino acids. The majority of hydrophobic moieties are buried inside the quaternary or tertiary structure due to a hydrophobic effect, and the majority of hydrophilic moieties are on the surface, free to participate in protein-water interactions. 'Salting-in' of proteins typically occurs at low salt levels, where the ions act to increase order of the protein's hydration layers and promote protein-water interactions (Philip, 1907; Glasstone et al., 1926; Glasstone et al., 1927; Eucken and Hertzberg, 1950; Desnoyers and Jolicoeur, 1969; Grover and Ryall, 2005). However, at high levels of salt, hydration layers can be disrupted as ion-water interactions become favored over protein-water interactions in a 'salting-out' process (Philip, 1907; Glasstone et al., 1926; Glasstone et al., 1927; Eucken and Hertzberg, 1950; Desnoyers and Jolicoeur, 1969; Grover and Ryall, 2005). As the ions attract water molecules away from the surface of the proteins, protein-protein aggregation is favored due to hydrophobic interactions. Aggregates continue to grow in size and number until they fall out of solution as a precipitate. The ability of ions to 'salt-in' or 'salt-out' proteins depends on both the ionic strength and type of cations and/or anions present, as described according to the Hofmeister series (Hofmeister, 1888) (Figure 2.1). Figure 2.1 below describes various anions and cations in decreasing order of their ability in precipitation and stabilization of proteins.

Anions:	$SO_4^{2-} > HPO_4^{2-} > acetate^- > Cl^- > NO_3^-$
Cations:	$N(CH_3)_4^+ > NH_4^+ > Na^+ = K^+ > Li^+ > Mg^{2+}$

Figure 2.1. Relative protein precipitation ability of cationic and anionic salts (Hofmeister, 1888).

Salts formed between cations and anions with higher precipitation ability in the series decrease the solubility of non-polar amino acids, favoring hydrophobic interactions to 'salt-out'

proteins. On the contrary, salts formed between cations and anions with lower precipitation ability in the series weaken the hydrophobic interactions and result in increasing solubility of non-polar amino acids, thus favoring the 'salting-in' process (Nostro and Ninham, 2012). Broadly speaking, ammonium sulfate $(NH_4)_2SO_4$ and sodium chloride (NaCl) are the most commonly used salts for research purposes (Paredes-Lopez et al., 1991; Alsohaimy et al., 2007; Sun and Arntfield, 2010; Can Karaca et al., 2011). Typically in the salt extraction procedure, proteins are initially dissolved in an aqueous NaCl solution (0.3-0.5 M) (Paredes-Lopez et al., 1991; Sun and Arntfield, 2010) at neutral pH, followed by a clarification procedure to remove insoluble material. Precipitation of the protein can be triggered by either diluting the supernatant with water to lower the ionic strength or by dialysis to remove the salts, resulting in the formation of protein micelles which grow in size and number until precipitation ensues. Alsohaimy et al. (2007) prepared protein isolates from chickpea, lupin and lentil using isoelectric precipitation (IEP) and ammonium sulfate precipitation. For all of these legumes, the latter method resulted in higher protein content (chickpea-90.6%, lupin-92.6% and lentil-93.0%) in comparison to the former method (chickpea-81.4%, lupin-87.3% and lentil-80.0%). On the contrary, Can Karaca et al. (2011) produced isolates from chickpea, faba bean, pea and lentil using IEP and salt extraction method and found that the protein levels obtained using the IEP method (chickpea-85.4%, faba-84.1%, pea-88.8%, and lentil-81.9%) were found to higher than the ones produced by the salt extraction method (chickpea-81.6%, faba-82.0%, pea-81.1%, and lentil-74.7%) (Can Karaca et al., 2011).

2.4 Functional properties of legume proteins

Protein flours, concentrates and isolates can be incorporated into various foods to increase their nutritional value and/or to provide specific and desirable functional attributes (Boye et al., 2010a). These functional attributes may include solubility, gelation, emulsifying ability, oil and water absorption capacity, and foaming. Moreover, functional properties of legume proteins contribute an important aspect in determining the competitiveness of the protein ingredient or the product in the market, as they can impact the sensory, physical and chemical properties of a food, which includes texture and organoleptic characteristics. In the literature, the functional attributes of legume proteins vary considerably due to differences in the raw material, processing, extraction methods and environmental conditions used during testing.

a) Solubility

Protein solubility plays a major role in various food applications as a number of functional properties such as foaming, gelation or thickening, and emulsification are closely related and often dependent on protein solubility. A high protein solubility may be helpful in producing food products such as beverages, infant milk powder, imitation milk and other products which require instant solubility with no residues left. For instance, imitation milk produced using lentil protein isolate reported to have same quality as compared to those prepared from soy protein isolate, however having a lower quality when formed using a pea protein isolate (Swanson, 1990). The solubility of protein depends on various attributes including hydrophobic/hydrophilic balance of the protein molecule (mainly the surface composition: polar/non polar amino acids), isoelectric point, pH, temperature, ionic strength and the type of ions present in the solution (Kiosseoglou and Paraskevopoulou, 2011). Proteins exhibit minimum solubility at their isoelectric point (pI) because of a zero net surface charge, resulting in aggregation of protein molecules into some larger structures, followed by precipitation. On the contrary, when the pH values are greater or less than the protein's pI, proteins exert a positive or negative net charge into solution, repelling one another to maximize solubility.

The solubility profile of concentrates and isolates from various pulses obtained by IEP or UF were found to be lowest between pH 4 and 6, and significantly increases with pH shifting to either more acidic or alkaline conditions (Kiosseoglou and Paraskevopoulou, 2011). Boye et al. (2010b) reported that the solubility of pea, chickpea and lentil protein concentrates, which were processed using IEP and UF/DF (diafiltration) techniques, were highest at pHs 1-3 and pHs 7-10. Moreover, the solubility profile varied with different varieties where, UF-yellow pea and UF-red lentil concentrates had the highest solubility at neutral pH, while at pH 3 and 8-10 solubility was highest for only UF-red lentil. In both cases, the lowest solubility was found for UF-chickpea (desi). The study by Can Karaca et al. (2011) on five different legumes (pea, chickpea, faba bean, lentil and soybean) showed higher overall solubility (determined at neutral pH) of these legume isolates prepared by the IEP method (85.9%) as compared to ones prepared by a salt extraction method (61.5%). For the IEP method, the pea protein isolate had the lowest solubility (61.4%); soybean isolates had the highest solubility (96.5%); and pea, lentil and chickpea isolates exhibited intermediate solubility (>90.0%). However, highly variable results were obtained for the solubility of salt-extracted isolates with values of 30.1% and 96.6% for chickpea

and soybean respectively, while intermediate solubility was observed for lentil (89.8%), pea (38.1%), and faba bean (52.5%). Solubility profile of isolates produced from kabuli (PBG-1, PDG-4, PDG-3, GL769 and GPF-2) and desi chickpea cultivars (L550) were found to be non-significant as a function of genotype ($p>0.05$) (Kaur and Singh, 2007). However, in the study of Barac et al. (2010), the solubility profile of six pea genotypes (Maja, Calvedon, Miracle, L1, L2 and L3) were found to be significantly different from each other except L2 and Maja ($p<0.05$).

b) Oil holding and water hydration capacities (OHC, WHC)

Oil holding capacity (OHC) and water holding capacity (WHC) refer to the extent to which oil and water, respectively, can be bound per gram of the protein material or legume flour (Boye et al., 2010a; Kiosseoglou and Paraskevopoulou, 2011). These properties are essential with respect to maintaining the quality of a product, its shelf life and consumer acceptability (texture and mouth feel). The ability of a protein to bind oil and water is important in preventing cook loss or leakage from the product during processing or storage (Kiosseoglou and Paraskevopoulou, 2011). Failure of a protein to bind water could lead to brittle and dry characteristics of the product (Boye et al., 2010a). WHC values for pulse protein concentrates, such as pea, faba bean, lentil and chickpea, have been determined by various groups (Fernandez-Quintela et al., 1997; Kaur and Singh, 2007; Boye et al., 2010b) to fall in the range of 0.6 to 4.9 g/g, suggesting that both pulse genotype and type could impact values. For instance, Kaur and Singh (2007) found that protein isolates prepared by kabuli chickpea cultivars (PBG-1, PDG-4, PDG-3, GL769 and GPF-2) produced significantly lower WHC than desi chickpea (L550) ($p<0.05$) which clearly indicates the impact of different cultivars in assessing functionality. Boye et al. (2010b) reported that for all the legumes studied (red and green lentil, desi and kabuli chickpea, yellow pea) IEP protein concentrates had higher WHCs than did ones prepared by UF (with the exception of red lentil protein concentrates) although no substantial differences were observed between WHC values of pulse concentrates prepared by either IEP or UF. The yellow pea concentrate (IEP) had the highest WHC value which was much higher than those of the kabuli and desi chickpea concentrates (IEP and UF) indicating the more significant effect of pulse type compared to extraction method on WHC.

Oil holding capacity (OHC) values reported by various authors (Parades-lopez et al., 1991; Fernandez-Quintela et al., 1997; Kaur and Singh, 2007) for different pulses range from

1.0-3.96 g/g, and seem to depend again on the type and variety of pulse used, and the method of preparation of the protein product. Boye et al. (2010b) studied the UF and IEP concentrates produced from red and green lentil, yellow pea and kabuli and desi chickpea. They reported that pulse variety and processing conditions had a larger impact on the OHC of yellow pea, kabuli chickpea and red lentil concentrates as compared to those made from desi chickpea and green lentil. Moreover, UF concentrates made from yellow pea, red lentil and kabuli chickpea had significantly higher OHC than their corresponding IEP concentrates. Red lentil and yellow pea concentrates produced by UF had highest OHC of 2.26 g/g and 1.17 g/g respectively. However, no significant differences in OHC were observed between the IEP produced concentrates ($p>0.05$) (Boye et al., 2010b). In the study of Kaur and Singh (2007), chickpea protein isolates were reported to have higher OHC than the corresponding flour samples. Moreover, in contrast to WHC, the OHC of kabuli chickpea was reported to be significantly higher than desi cultivars ($p<0.05$).

The water and oil holding properties of legume proteins may be essential in formulation of food products such as meat, pasta, cookies, etc. In producing low fat meat products, water is added to substitute the fat loss. And, water holding compounds are added to prevent cooking losses and meat shrinkage which includes proteins (whey, soy and collagen), lipids (soy lecithin) and carbohydrates (flours, starches and gums) (Brewer, 2012). For instance, soy proteins added to ground beef improves the tenderness, moisture retention, decreases cooking losses, and inhibits rancidity (Kotula and Berry, 1986). Deliza et al. (2002) replaced meat in ground beef mixture with hydrated textured soybean protein (15 or 30%) and found that beef patties were more tendered as compared to controls, although the overall flavour quality is reduced with having less beefy flavour. However, legumes (navy beans, chickpeas, mung beans and, red kidney beans) when substituted at a level of 15% in beef mince resulted in acceptable products, with chickpea preferred over other legumes (Muller and Redden, 1995).

c) Emulsification

An emulsion is a mixture of two or more immiscible liquids (usually oil and water), where one of the liquids (the dispersed phase) is mixed in to other (the continuous phase) in the form of small spherical droplets (McClements, 2005). Emulsions are generally classified into two types: oil-in-water (O/W), in which oil droplets are dispersed within an aqueous phase (e.g.,

milk, mayonnaise, cream and soups); or water-in-oil (W/O), in which water droplets are dispersed within an oil phase (e.g., butter and margarine). Emulsions are thermodynamically unstable and with time separate into oil and liquid layers due to collision and coalescence of droplets (McClements, 2005). Stabilizers such as emulsifiers can be used to produce stable emulsions. For instance, protein as an emulsifier acts by adsorbing onto the oil-water interface to form a viscoelastic film surrounding the oil droplets. Stability is enhanced through electrostatic charge repulsion (depending on the pH), steric hindrance or increases to the continuous phase viscosity (McClements, 2005).

Protein emulsifiers are used worldwide because of their ability to adsorb at the droplet surface in an O/W emulsion during the process of homogenization, thereby reducing interfacial tension. The adsorbed protein molecules present at the surface act as a separating membrane preventing coalescence with the neighbouring droplets (Kiosseoglou and Paraskevopoulou, 2011). To be an effective emulsifier, protein must exhibit the following properties: fast adsorption at the oil-water interface, ability to form a protective and cohesive layer around the oil droplets, and ability to unfold at the interface (Damodaran, 2005). Various studies reported that the emulsifying ability of legume protein concentrates or isolates are dependent on the type of legume or the method (IEP/UF/salt extraction) used in their preparation. For instance, Fuhrmeister and Meuser (2003) reported that a pea protein isolate prepared by an IEP method was found to have lower emulsifying ability as compared to one prepared using UF.

Emulsion activity index (EAI) refers to the area of emulsion stabilized per gram of emulsifier or protein material and expressed as m^2/g whereas emulsion stability index (ESI) refers to the measure of stability of this emulsion as a function of time. Emulsion capacity (EC) is amount of oil homogenized per gram of protein material and expressed as g oil/g protein whereas creaming stability (CS) is the ability of emulsion to resist creaming and formation of serum layer as the time passes, and measured as %. The study conducted by Can Karaca et al. (2011) on different legumes (pea, chickpea, faba bean, soybean and lentil) showed that both legume source and extraction method (IEP or UF) had significant effects on emulsifying and physicochemical properties. Both EAI and ESI were significantly affected by legume source and extraction method, whereas EC was dependent on the legume source only. However, Boye et al. (2010b), studying the functional properties of chickpea, lentil and pea protein concentrates, concluded that IEP and UF preparation methods had little impact on emulsifying properties.

Barac et al. (2010) studying functional properties of six pea genotypes reported significant differences in emulsifying properties (EAI and ESI) as a function of genotypes and also as a function of pH for the six same genotypes. The EAI of pea genotypes tested in this study was significantly higher than the commercial pea protein isolates.

Emulsifying and other functional properties of proteins can also be improved with protein modifications such as limited enzymatic hydrolysis using proteases (e.g. trypsin). The hydrolysis reaction results in partial unravelling of protein molecules thus, exposing more ionic and hydrophobic groups for interaction with oil droplets (Panyam and Kilara, 1996). For instance, trypsin treated oat bran protein with a ~4-8% degree of hydrolysis (DH) found to have improved solubility, water holding, foaming and emulsifying properties as compared to those of native proteins (Guan et al., 2007). On the contrary, Avramenko et al. (2013) reported detrimental effects of trypsin mediated hydrolysis (DH~4-20%) of lentil protein isolates. Here, except zeta potential, all the physicochemical properties (surface hydrophobicity and interfacial tension) and emulsifying properties (emulsion activity and stability indices) were found to have lower values as compared to the unhydrolysed lentil protein isolate. This suggests that processing conditions might have specific effects dependent on protein source.

Legume proteins play a vital role in formulation of a number of novel foods (such as sausages, bologna, meat analogues, cakes and soups) by formation and stabilisation of emulsions. Meat analogues are foods which are made from nonmeat ingredients, structurally similar to meat and may have same texture, flavour, appearance, and chemical characteristics (Malav et al., 2015). Some of the traditional foods such as wheat gluten, rice, mushrooms, tofu and legumes when added with flavours mimic the finished meat products such as chicken, beef, sausage etc. (Malav et al., 2015). Soybean protein is an important meat analogue since it has meat like texture and provides similar amino acid profile as with meat proteins (Malav et al., 2015). Tofu is a widely consumed meat analogue made from soy, which provides a good source of protein, calcium and, iron. In general, the market for meat analogues is huge which includes vegetarians, vegans, and people who do not eat meat products because of religious or cultural practices.

d) Foaming

Similar to emulsions, foams also have two immiscible phases (aqueous and gas), and

require an energy input to facilitate their formation. Foams are comprised of a dispersed gas phase within a continuous aqueous phase (Damodaran, 2005). Proteins in solution adsorb to the gas-liquid interface in a similar manner as in emulsions to form a viscoelastic film surrounding the gas bubbles that helps resist rupturing and bubble fusion (Kiosseoglou and Paraskevopoulou, 2011). In contrast to emulsions, the major driving mechanism associated with foam instability is associated with Oswald ripening, which involves the diffusion of small gas bubbles through the continuous phase in order to become absorbed into a larger gas bubble (Damodaran, 2005). Rupture of the viscoelastic film leads to drainage of the continuous liquid phase through the film matrix. Various food products are available which use protein as a stabilizer including meringues, whipped desserts, mousses and leavened bakery products (Townsend and Nakai, 1983). Vose (1980) reported that the foaming properties of faba bean and yellow pea isolates, prepared using UF, were higher than that of skim milk powder, wheat flour and soy protein isolates. A faba bean isolate was observed to have better foaming properties than pea protein isolate.

Foaming capacity (FC) refers to the volume of foam generated after homogenization of certain amount of protein solution whereas foam stability (FS) refers to the ability to retain foam structure and resistance in formation of serum layer as a function of time. In the study of Sathe and Salunkhe (1981) on great northern bean (*Phaseolus vulgaris* L.) protein materials, the FCs were in the following increasing order: albumins (180%) > protein concentrate (164%) > globulins (140%) ~ egg albumin (140%) > flour (132%) > isolate (106%), where egg albumin was the standard for measuring foaming capacity. These results indicated that, all great northern bean protein materials, except the isolate, had FCs that was comparable to or higher than that of egg albumin. However, the foaming stabilities were not up to the mark with egg albumin, and hence the overall foaming ability was given only a fair mark (Sathe and Salunkhe, 1981; Boye et al., 2010a). Boye et al. (2010b) studied and compared the functional properties of yellow pea, green and red lentil, and kabuli and desi chickpea protein concentrates prepared using IEP and UF techniques. In their studies, they found that foaming capacity (which ranged from 98% to 106%) was similar for pea and lentil protein concentrates irrespective of extraction method used. However, the desi and kabuli chickpea concentrates prepared by the IEP method showed higher foaming capacity than for others. In general, it was observed that chickpea showed higher foaming capacity and expansion but lower foam stability as compared to other sources.

Furthermore, variability was observed in foaming stability with kabuli and desi chickpea and green lentil concentrates prepared by the IEP method, which showed higher foam stability values compared to concentrates prepared by the UF method. Barac et al. (2010) studying the functional properties of isolates produced from six pea genotypes using the IEP method, reported significant differences in their foaming properties as a function of genotype and regardless of changes in pH. Generally, a low foam stability was observed probably because of use of low concentration of protein in formation of protein solution. However, foaming capacity was highest for Maja cultivar, which was significantly higher than the commercial pea protein isolate.

2.5 Faba bean (*Vicia faba* L.)

Vicia faba L. is a key and inexpensive food crop in developing countries like Egypt, India, Pakistan and China and is used for human consumption, animal feed and for agronomic practices (Kopke and Nemecek, 2010; Vioque et al., 2012). Faba bean like other legumes fixes atmospheric nitrogen in symbiosis with bacteria (*Rhizobium leguminozarum*) allowing for less fertilizers to be used during production (El Fiel et al., 2002). Moreover, faba bean helps in reducing root rot and gangrene, and in improving the physical and chemical properties of soil (Micek et al., 2015). The yield of faba beans under favourable conditions may reach up to 4 tonnes per hectare, and typically is used for feed purposes to provide nutrition of adult farm animals (Micek et al., 2015). For instance, Strzetelski et al. (1996) reported that faba bean meal can be used in place of soybean protein meal for young calves without having any negative impact on feed utilisation and weight gains. Faba bean contains high amounts of carbohydrate (57.3%) (Reddy et al., 1985; Oke et al., 1995; Frank-Peterside et al., 2002; Ofuya, 2002) and protein (24.0-36.0%) (Amartiefo et al., 2002; Frank-Peterside et al., 2002; Ofuya, 2002; Can Karaca et al., 2011; Coda et al., 2015), and is also a valuable source of minerals (Ca^{2+}, Mg^{2+} and Fe^{2+}) and vitamins (thiamine, riboflavin and pyridoxine) (Ofuya and Akhidue, 2005). Faba bean seeds have a very low level of fat (< 2%) (Cerning et al., 1975), and have crude fibre content of ~2.4% (Gueguen, 1983).

Similar to most of the legumes, the major proteins of faba bean seed can be divided into globulin and albumin fractions, where globulins account for the main storage proteins (60%) while the albumins account (20%) for most of the regulatory proteins (metabolic activities and enzymatic regulations) (Makri et al., 2005). The structure of 11S and 7S proteins in faba bean is

similar to that in other pulses, as described earlier. A large molecular heterogeneity was observed in legumin and vicilin proteins of faba bean flour samples (Tucci et al., 1991). Faba bean 11S proteins have large proportions of arginine, glutamic acid and aspartic acid, but are low in methionine and cysteine (Lampart-Szczapa, 2012). Faba bean vicilin contains a variety of polypeptide fragments (15 to 66 kDa) and are also glycosylated, unlike pea vicilin (Lampart-Szczapa, 2012).

The protein profile of faba bean seeds calculated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is similar to that of pea (Nikolic et al., 2012). The major bands of faba bean storage proteins under reducing conditions correspond to the following proteins: convicilin (~74 kDa), vicilin (~50 kDa), α-legumin (~36 kDa) and β-legumin (~24 kDa) (Nikolic et al., 2012). However, there could be slight variations in molecular weight of these bands subject to environmental or agronomical conditions. In the study of Vioque et al. (2012), the faba bean protein isolates produced by alkaline extraction/IEP (Na_2SO_3, pH-10.5, protein precipitation at pH 4.0) yielded a protein content of more than 90% (Table 2.1). However, lipids, sugar and polyphenol content were found to be negligible in the isolates (Table 2.1). A very high dietary fibre content of 57% was also obtained in the alkaline solid residue formed after the alkaline extraction step in isolate formation (Vioque et al., 2012) (Table 2.1).

Table 2.1. Approximate chemical composition of faba bean flour, protein isolate, and alkaline residue. All measurements are in % (w/w) (Vioque et al., 2012).

Parameters	Flour	Protein isolate	Alkaline solid residue
Ash	4.1	3.2	1.7
Fibre	31.3	n.d.[a]	57.0
Protein	26.6	92.4	6.9
Lipids	1.8	Tr[b]	1.0
Soluble sugars	0.7	Tr[b]	0.1
Polyphenols	0.2	Tr[b]	Tr[b]
Carbohydrates	35.4	4.4[c]	33.2

[a] Not determined, [b] Traces (less than 0.1%), [c] Including fibre.

Faba bean like other legumes contains anti-nutritive factors (ANFs) including pyrimidine glycosides called vicine and convicine, condensed tannins, protease inhibitors, alkaloids, lectins, and others (Liener, 1990). The content of these ANFs can be reduced using different methods such as dehulling, soaking, air classification, wet extraction, extrusion or heat treatment (Van der Poel, 1990; Jezierny et al., 2010). Faba beans containing vicine and convicine were found to be 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 (Robblee et al., 1977; Campbell et al., 1980; Davidson, 1980; Wang and Ueberschar, 1990), while in humans they are known for causing favism (a type of haemolytic anemia) (McMillan et al., 2001; Vioque et al., 2012). However, their content can be reduced to a negligible amount via isolate preparation as shown in the study of Vioque et al. (2012), where faba bean isolates produced by alkaline extraction (Na_2SO_3, pH-10.5, protein precipitation at pH 4.0) were analyzed for vicine and convicine content using Reverse Phase High Performance Liquid Chromatography (RP-HPLC). These were found to contain less than 1% of these glycosides in comparison to the original flour (Vioque et al., 2012).

Modifications at genetic level have also showed to reduce the level of tannins and/or vicine and convicine content in faba beans (Crepon et al., 2010). Micek et al. (2015) found that low tannin varieties of faba bean had a higher crude protein and lower fibre content, and a significantly higher invitro digestibility ($p<0.01$). Some of the biological methods such as germination, enzymatic treatments, and fermentation have also been utilized to reduce the ANFs levels (Alonso et al., 2000; Granito et al., 2002; Luo et al., 2009). For instance, fermentation of faba bean flours by *Lactobacillus plantarum* VTT E-133328 resulted in a decrease in more than 90% of vicine and convicine content, and a significant reduction in trypsin inhibitor activity and in the level of condensed tannins (Coda et al., 2015). Fermentation also resulted in an increase in level of free amino acids and improved protein digestibility (Coda et al., 2015).

Faba bean have been studied and found to be comparable to those of other pulses (Andersson et al., 1985; Zheng et al., 1992; Fernandez-Quintela et al., 1997; Cepeda et al., 1998; Galazka et al., 1999; Vioque et al., 2012). Faba bean isolates produced using the IEP method were found to have higher functional properties (solubility, emulsion capacity, creaming stability and emulsion activity and stability indices) than pea protein isolates and comparable to those produced by chickpea and lentil protein isolates (Can Karaca et al., 2011). For instance, a

solubility of >90% and emulsion capacity of ~513 g oil/g protein was reported for faba bean protein isolate compared to solubility of just ~62% and emulsion capacity of ~477 g oil/g protein for pea protein isolate (Can Karaca et al., 2011). Attempts were also made to improve the functional properties of faba bean using various protein modification mechanisms such as acetylation and succinylation (Knopfe et al., 1998; Schwenke et al., 1998).

Similar to other pulses, the chemical, functional or physicochemical properties of faba beans could be influenced by certain factors including processing, extraction or environmental. Variance in genotypes or environmental factors which accounts for soil conditions, precipitation, location, year of growth etc. may play a restrictive role in picking up stable faba bean lines and hence in producing products with uniform consistency. For instance, Hood-Niefer et al. (2011) reported the differences in protein and starch concentrations, and physicochemical properties of starch for 10 pea and 11 faba bean genotypes grown at various locations in Saskatchewan, Canada, for the year 2006 and 2007. Here, the effect of genotype for both pea and faba bean was found to be substantial while the effect of environment (location and year) was not. However, a significant interaction was found between location and genotype for protein concentration in pea, and starch concentration in faba bean. In a study by Bhatty (1974), 12 faba bean cultivars obtained from University of Saskatchewan experimental plots (Saskatoon, SK) were analyzed for chemical composition differences. Here, the protein content varies from 26 to 35% however, fibre, ash and lipid content were found to be uniform in nature (Bhatty, 1974). More research studies concentrating on these factors are required since improved functional properties of faba bean isolates or concentrates would help food industries in the formation of varied and distinct food products with desirable functional attributes at sustainable prices. Furthermore, this inexpensive source of protein and other nutrients could be blended with other foods, especially in developing countries, such that the nutritional requirements could be met in a cost effective manner.

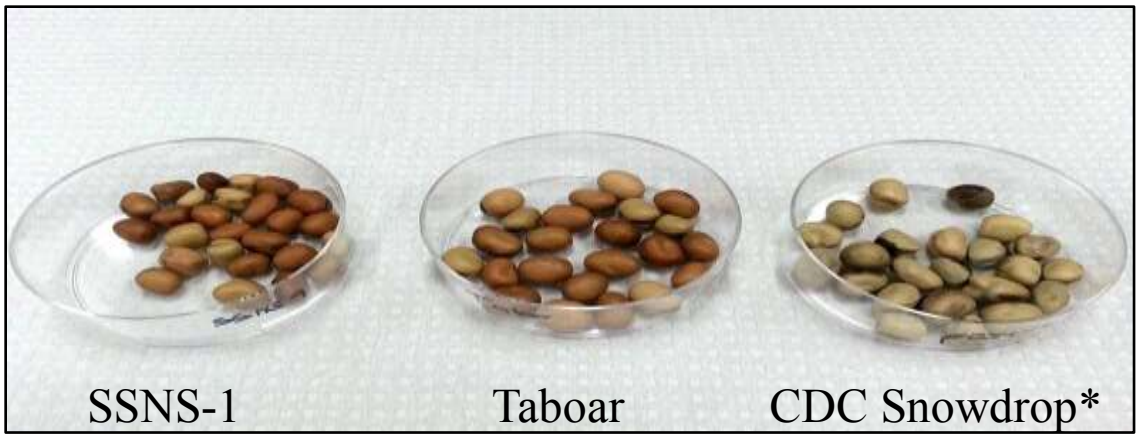
3. MATERIALS AND METHODS

3.1 Materials

Seed samples of seven faba bean (*Vicia faba* L.) genotypes (CDC Fatima, Taboar, SSNS-1, FB9-4, FB18-20, Snowbird, CDC Snowdrop) grown in 2011 and 2012 at locations within Canada [Manitoba (Arborg and Melita), Alberta (Lacombe and Edmonton), and Saskatchewan (Saskatoon-Sutherland, Saskatoon-Preston, Outlook, Rosthern, and Meath Park)] were kindly donated by the Crop Development Centre at the University of Saskatchewan (Saskatoon, SK, Canada) (Table 3.1). Faba bean genotypes low in tannin has been reported to have a positive impact on nutritional value and protein digestibility (Crepon et al., 2010). In this study, Snowbird and CDC Snowdrop were the only zero tannin genotypes. All genotypes contained normal vicine levels. SSNS-1, CDC Snowdrop and Taboar were of small seed size, Fatima and Snowbird were of intermediate seed size, and FB18-20 and FB9-4 were of large seed size (Figure 3.1). The meteorological conditions and soil zones over locations where the faba bean seed was grown are shown in Table 3.2 and Figure 3.2 respectively. Considering the visual colour of seed samples, Snowbird and CDC Snowdrop seed was light in colour with no redness, FB9-4, FB18-20 and CDC Fatima seed had darker colour with slight redness, and SSNS-1 and Taboar seed were dark in colour with high redness.

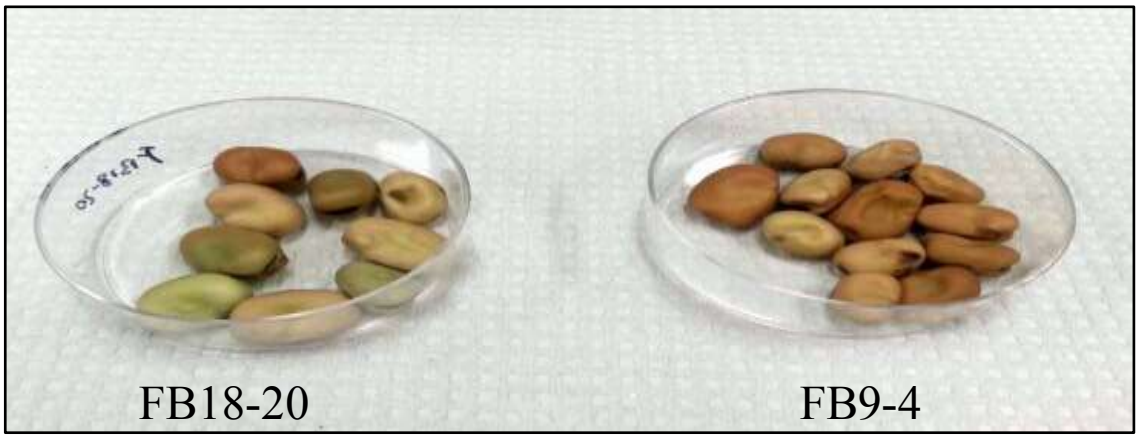
The following commercial isolates were used in this study: Propulse pea protein isolate (Nutri-Pea Limited, Portage La Prairie, MB, Canada), dried egg white (Ballas Egg Product Corporation, Zanesville, OH, USA), Prolisse soy protein isolate (Cargill, Wayzata, MN, USA), PROLITE 100 wheat protein isolate (Archer Daniels Midland Company, 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^{TM} water purification system (Millipore Corp., Milford, MA, USA).

(A) Faba bean genotypes with smallest seed size



(B) Faba bean genotypes with intermediate seed size

(C) Faba bean genotypes with largest seed size



*Genotypes having zero tannin content

Figure 3.1 Images of faba bean genotypes with (A) small, (B) intermediate (C) and large seed sizes.

Table 3.1 Seven faba bean genotypes grown at several locations within Saskatchewan, Alberta and Manitoba in 2011 and 2012 (climate.weather.gc.ca).

Genotype	2011		2012	
	Location	Location	Location	Location
CDC Fatima	Arborg (MB)	Lacombe (AB)	Outlook (SK)	Sask-P (SK)
Taboar	Arborg (MB)	Lacombe (AB)	Outlook (SK)	Sask-P (SK)
SSNS-1	Arborg (MB)	Edmonton (AB)	Outlook (SK)	Rosthern (SK)
FB9-4	Arborg (MB)	Edmonton (AB)	Outlook (SK)	Rosthern (SK)
FB18-20	Arborg (MB)	Edmonton (AB)	Outlook (SK)	Rosthern (SK)
Snowbird	Melita (MB)	Lacombe (AB)	Outlook (SK)	Sask-S (SK)
CDC Snowdrop	Sask-S (SK)	Edmonton (AB)	Meath Park (SK)	Rosthern (SK)

Sask-S and Sask-P represent Saskatoon-Sutherland and Saskatoon-Preston respectively.

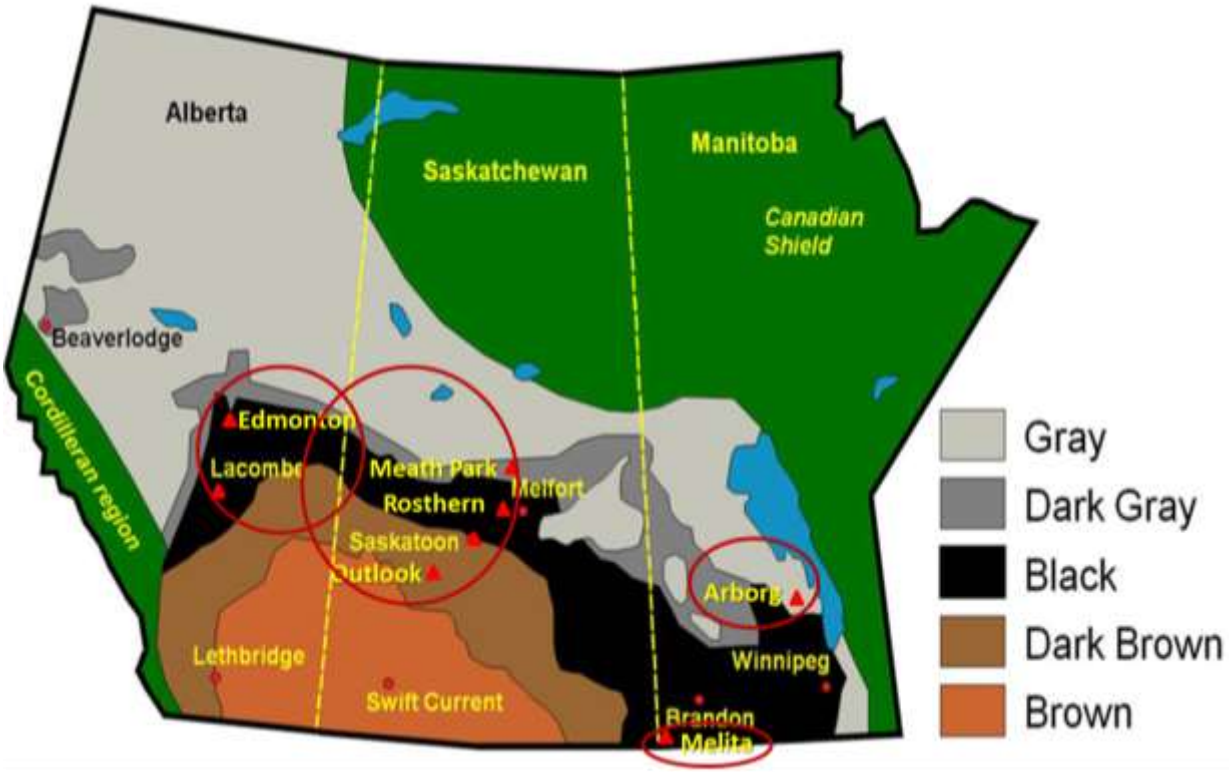


Figure 3.2 Major soil zones and locations in the prairie region (climate.weather.gc.ca).

Table 3.2 Average monthly temperature and precipitation data for April to October over a two year duration (2011–2012) at locations where faba bean seed was grown within Saskatchewan, Alberta and Manitoba (climate.weather.gc.ca).

Locations	**Soil zone**	**2011 / 2012**	
		Average monthly temperature (°C)	**Average monthly precipitation (mm)**
Saskatoon, SK	Dark Brown	12.3 / 11.7	34.2 / 56.5
Outlook, SK	Dark Brown	12.7 / 12.2	39.6 / 56.6
Rosthern, SK	Black	NA	NA
Meath Park, SK	Dark Grey	NA	NA
Lacombe, AB	Black	10.5 / 10.8	53.8 / 44.5
Edmonton, AB	Black	12.4 / 12.5	46.3 / 44.6
Arborg, MB	Grey	13.3 / 12.9	46.9 / 73.1
Melita, MB	Black	13.4 / 13.2	57.7 / 32.3

3.2 Processing of faba bean seed

Prior to protein isolate preparation, all seed was 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 into finer flour using a UDY Cyclone Sample Mill (UDY Corporation, Fort Collins, CO, USA). All flours were defatted according to Can Karaca et al. (2011) 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 times. The subsequent mixture was then filtered using Whatman #1 filter paper (Whatman International Ltd., Maidstone, UK) followed by overnight air drying in a fume hood. Dried defatted flour was stored at 4°C until used.

3.3 Preparation of faba bean protein isolates

Faba bean protein isolates were prepared by alkaline extraction followed by isoelectric point precipitation according to Makri et al. (2006). 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 centrifugation (1600 x *g*, 20 min, 4°C) using a Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC, USA). The supernatant was collected and the pellet was re-suspended in water (1:5, w/v), stirred and then centrifuged (1600 x *g*, 20 min, 4°C). The supernatants were combined, the pH adjusted to 4.5 using 1M HCl, and then centrifuged (1600 x *g*, 20 min, 4°C). The recovered pellet was washed using deionized water gently poured on the surface using a transfer pipette, adjusted to pH 7.0 using 1M NaOH, stored at -30°C, and later freeze dried to obtain the protein isolate.

3.4 Proximate analysis

Proximate analysis of all flour materials was carried out according to Association of Official Analytical Chemists (AOAC) methods 925.10, 923.03, 920.85 and 997.09 for moisture, ash, crude fat and crude protein (%N x 6.25), respectively (AOAC, 2003). For isolates, only protein, ash and moisture contents were reported since the crude fat level in isolates was observed to be negligible due to the prior defatting step. Ash, fat and protein levels were reported on a percent dry weight basis (d.b.). All proximate analyses were reported as mean ± one standard deviation (n = 2).

3.5 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 proportion of legumin and vicilin [(reported as the legumin:vicilin (L/V) ratio))]. Separation of legumin and vicilins fractions was carried out via SDS-PAGE according to Laemmli (1970) with some modifications. In brief, 75 µL of a 3 mg/mL flour-water (or 1 mg/mL isolate-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 was 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 x *g* for 5 min. Pre-cast 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 200V using a Power Source 300V Electrophoresis Power Supply (VWR, Mississauga, ON, Canada). Precision Plus Protein™ Prestained Standards (BIO-RAD) ranging from 10 KDa 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 de-staining with methanol: water: acetic acid (3:6:1) three times over a 24 h period. The de-stained 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) (Rasband, 1997). All samples were run in triplicate. The L/V ratio was reported as the mean ± one standard deviation (n = 3).

3.6 Colour

The colour of the flours and isolates was determined according to the method of Kaur and Singh (2007) based on the determination of *L*, a* and b** values. *L** is the brightness, 0 to 100, moving from dark to light; *a** is red-green, with red associated with higher values of a*; *b** is yellow-blue, with yellow associated with higher values of b*. L_s, a_s and b_s are the standard reference values used for calibration of the instrument. All measurements are reported as the mean ± one standard deviation (n = 3).

3.7 Physicochemical properties

For physicochemical property testing, protein isolate 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).

3.7.1 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:

$$U_E = \frac{2\varepsilon \times \zeta \times f(\kappa\alpha)}{3\eta} \quad \text{(eq. 3.1)}$$

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

3.7.2 Intrinsic fluorescence

Intrinsic fluorescence of 0.05% (w/w) protein isolate solutions was measured to give an estimate of the 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 a range from 285 to 450 nm with a slit width of 5 nm in 0.5 nm increments. 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. All measurements are reported as the mean ± one standard deviation (n = 2).

3.7.3. Interfacial tension

Interfacial tension between 0.25% (w/w) protein isolate solutions and canola oil and between protein solutions and the air interface were determined using the Du Noüy ring and a semi-automatic tensiometer (Lauda TD2, GmbH and 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}).

$$\gamma = \frac{F_{max}}{4\pi R\beta} \quad \text{(eq. 3.2)}$$

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 the mean ± one standard deviation (n = 2).

3.8 Functional properties

For functional property testing, protein isolate solutions of various concentrations were prepared by dispersing the protein 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.).

3.8.1 Oil holding capacity

Oil ho1lding capacity (OHC) was determined according to Chakraborty (1986) with slight modifications. Ten millilitre of canola oil was combined with 1 g of protein isolate (weight 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 decanting of the supernatant. The OHC was calculating according to eq. 3.3:

$$OHC = \frac{Wt_{Wet} - Wt_{Dry}}{Wt_{Dry}} \times 100\% \qquad \text{(eq. 3.3)}$$

where Wt_{Wet} and Wt_{Dry} are the weights of the wet and dry samples, respectively. All measurements are reported as the mean ± one standard deviation (n = 2).

3.8.2 Foaming capacity and stability

Foaming capacity (FC) and stability (FS) for 1.0% (w/w) protein isolate solutions were determined according to Liu et al. (2010). Fifteen millilitre of each protein solution was transferred to a 400-mL beaker for homogenization using 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 time 0 and at 30 min, with FC and FS determined using eq. 3.4 and 3.5, respectively:

$$FC = \frac{V_{F0}}{V_{sample}} \times 100\% \qquad \text{(eq. 3.4)}$$

$$FS = \frac{V_{F30}}{V_{F0}} \times 100\% \qquad \text{(eq. 3.5)}$$

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 the mean ± one standard deviation (n = 2).

3.8.3 Emulsion capacity

Emulsion capacity (EC) was determined according to Can Karaca et al. (2011) with slight modifications. Two gram of a 1.0% (w/w) protein isolate solution was homogenized using Macro Homogenizer (Omni International) at speed 4 (~7,200 rpm) for 5 min, with various quantities of canola oil (2-5 g). After the sample was homogenized, the conductivity of the emulsion was measured using an Orion 3-Star bench top conductivity meter (Thermo Scientific, Waltham, MA, USA) with a four electrode conductivity cell until the observance of an inversion point (a swift 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 g of protein before and after the inversion point. All measurements were reported as the mean ± one standard deviation (n = 2).

3.8.4 Creaming stability

Creaming stability (CS) of 1.0% (w/w) protein isolate solutions was measured by homogenizing 5 mL of protein solution with 5 mL of canola oil using 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 emulsion after 30 min. The creaming stability was calculated using eq. 3.6:

$$CS = \frac{V_B - V_A}{V_A} \times 100\% \qquad \text{(eq. 3.6)}$$

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

3.8.5 Emulsion activity and stability indices

Emulsion activity (EAI) and stability (ESI) indices for 0.5% (w/w) protein isolate

solutions were determined according to the method of Pearce and Kinsella (1978). Fixed quantities of protein isolate solution (5 g) and canola oil (5 g) were homogenized using 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-µL 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, Madison, WI, USA) using plastic cuvettes (1-cm path length). Similarly, another 50-µL aliquot of emulsion was taken after 10 min and the absorbance was measured using the procedure above. EAI and ESI were calculated using the following equations:

$$EAI = \frac{2 \times 2.203 \times A_0 \times n}{c \times \varphi \times 10000} \quad \text{(eq. 3.7)}$$

$$ESI = \frac{A_0}{\Delta A} \times t \quad \text{(eq. 3.8)}$$

where EAI (m^2/g) is expressed as the area of interface (m^2) stabilized per unit weight of protein (g), whereas 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 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). All measurements are reported as the mean ± one standard deviation (n = 2).

3.8.6 Protein solubility

Protein solubility for a 1.0% (w/w) solution was determined according to Can Karaca et al. (2011) with slight modifications. In brief, 20 g of protein isolate solution was transferred to a 50-mL centrifuge tube followed by centrifugation (9100 x *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 by using 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 initial protein content of the original sample and multiplying by 100%. All measurements were reported as the mean ± one standard deviation (n=2).

3.9 Statistics

SPSS software (IBM Corporation, Armonk, NY, USA) was used for all of the statistical analysis in this study. Due to limited seed availability for some locations, analyzing an interaction effect between genotype and environment for all faba bean genotypes was not possible. Hence, a one-way analysis of variance (ANOVA) along with a post-hoc Tukey test was performed to evaluate the overall effect of genotype for all physicochemical and functional properties analyzed in this study. To evaluate the environmental differences within each genotype, a second one-way ANOVA was performed for the same parameters. A two-way ANOVA studying the interaction effects of genotype x location and genotype x year was conducted for only three genotypes (SSNS-1, FB9-4 and FB18-20) since they were grown at the same locations in 2011 and 2012. STATISTICA software (StatSoft, 2014) was used to generate a scatter plot evaluating the overall distribution of genotypes, years and locations in a principal component analysis (PCA) model. This plot was useful in analysing the comparative effects of different genotypes, years and locations based on their influence on the PCA model. A simple Pearson correlation (r) analysis was performed to identify any significant correlations between the functional properties, physicochemical properties, extraction yield, proximate composition, colour characteristics and L/V ratio of the faba bean protein isolates.

4. RESULTS

4.1 Proximate analysis

a) Faba bean flour

A one-way ANOVA revealed the effect of genotype on the percentage of protein, crude fat and ash in the flours to be non-significant ($p>0.05$). The average levels of moisture, protein, crude fat and ash in the faba bean flours were 6.7%, 31.8% (dry weight basis, d.b.), 1.3% (d.b.) and 3.4% (d.b.), respectively. However, some minor differences were evident within genotypes depending on the growing location and year. In the case of protein, CDC Fatima grown at Outlook in 2012 (31.3%) was found to have a significantly lower protein level than when grown at Saskatoon-Preston in 2012 (32.0%), Arborg in 2011 (33.3%) or Lacombe in 2011 (33.7%) ($p<0.01$) (Table 4.1). Flour from Taboar grown at Saskatoon-Preston in 2012 (30.0%) was found to be significantly lower in protein than when grown at Outlook in 2012 (31.9%), Arborg in 2011 (32.5%) or Lacombe in 2011 (33.7%) ($p<0.01$). Flour from SSNS-1 grown at Rosthern in 2012 (31.8%) was significantly lower in protein than when grown at Outlook in 2012 (32.2%), Edmonton in 2011 (32.8%) or Arborg in 2011 (33.2%) ($p<0.01$). For FB9-4, the protein level in flour when grown at Rosthern in 2012 (31.0%) was significantly lower than when grown at Outlook in 2012 (32.4%), Edmonton in 2011 (32.1%) or Arborg in 2011 (34.1%) ($p<0.01$). FB18-20 when grown at Edmonton in 2011 (30.4%) exhibited a significantly lower level of protein in flour than when grown at Rosthern in 2012 (30.6%), Outlook in 2012 (31.0%) or Arborg in 2011 (32.1%) ($p<0.01$). Flours from Snowbird grown at Melita in 2011 or Saskatoon-Sutherland in 2012 had similar protein levels (30.1%) ($p>0.05$), which were significantly lower than when grown at Outlook in 2012 (32.8%) or Lacombe in 2011 (33.9%) ($p<0.01$). The level of protein in flour from CDC Snowdrop was similar when grown at Rosthern in 2012 (30.0%) or Meath Park in 2012 (30.2%) ($p>0.05$) and was significantly lower than when grown at Edmonton in 2011 (32.6%) and significantly higher than when grown at Saskatoon-Sutherland in 2011 (27.3%) ($p<0.01$).

Table 4.1 Percent protein, crude fat and ash in faba bean flours produced from seven genotypes in two years at several locations in Western Canada. Data represent the mean ± one standard deviation (n = 2).

	Protein (%, d.b.)	Crude fat (%, d.b.)	Ash (%, d.b.)
a) CDC Fatima			
Arborg, MB (2011)	33.3 ± 0.1	0.9 ± 0.1	3.3 ± 0.2
Lacombe, AB (2011)	33.7 ± 0.0	1.5 ± 0.1	2.7 ± 0.2
Outlook, SK (2012)	31.3 ± 0.0	1.3 ± 0.0	4.1 ± 0.2
Saskatoon-Preston, SK (2012)	32.0 ± 0.1	1.3 ± 0.0	3.5 ± 0.1
b) Taboar			
Arborg, MB (2011)	32.5 ± 0.0	1.0 ± 0.1	2.9 ± 0.1
Lacombe, AB (2011)	33.7 ± 0.0	1.4 ± 0.0	3.4 ± 0.0
Outlook, SK (2012)	31.8 ± 0.0	1.2 ± 0.0	3.0 ± 0.0
Saskatoon-Preston, SK (2012)	30.0 ± 0.1	1.1 ± 0.0	3.0 ± 0.0
c) SSNS-1			
Arborg, MB (2011)	33.2 ± 0.0	0.9 ± 0.0	3.6 ± 0.3
Edmonton, AB (2011)	32.9 ± 0.1	1.2 ± 0.0	3.2 ± 0.0
Outlook, SK (2012)	32.2 ± 0.1	1.2 ± 0.0	2.8 ± 0.0
Rosthern, SK (2012)	31.8 ± 0.0	1.3 ± 0.0	3.0 ± 0.2
d) FB9-4			
Arborg, MB (2011)	34.1 ± 0.0	1.0 ± 0.0	3.2 ± 0.2
Edmonton, AB (2011)	32.1 ± 0.0	1.3 ± 0.0	3.1 ± 0.1
Outlook, SK (2012)	32.4 ± 0.1	1.3 ± 0.1	3.8 ± 0.3
Rosthern, SK (2012)	31.0 ± 0.0	1.5 ± 0.0	3.7 ± 0.2
e) FB18-20			
Arborg, MB (2011)	32.1 ± 0.0	1.2 ± 0,1	3.2 ± 0.2
Edmonton, AB (2011)	30.4 ± 0.0	1.3 ± 0.1	3.2 ± 0.0
Outlook, SK (2012)	31.0 ± 0.1	1.4 ± 0.0	3.8 ± 0.3
Rosthern, SK (2012)	30.6 ± 0.1	1.5 ± 0.1	3.7 ± 0.1
f) Snowbird			
Lacombe, AB (2011)	33.9 ± 0.1	1.4 ± 0.1	2.8 ± 0.0
Melita, MB (2011)	30.1 ± 0.1	1.2 ± 0.1	3.7 ± 0.0
Outlook, SK (2012)	32.8 ± 0.3	1.3 ± 0.0	4.3 ± 0.3
Saskatoon-Sutherland, SK (2012)	30.1 ± 0.1	1.5 ± 0.3	3.0 ± 0.0
g) CDC Snowdrop			
Edmonton, AB (2011)	32.6 ± 0.0	1.1 ± 0.0	3.1 ± 0.0
Saskatoon-Sutherland, SK (2011)	27.3 ± 0.1	1.3 ± 0.0	3.7 ± 0.2
Rosthern, SK (2012)	30.0 ± 0.0	1.3 ± 0.0	3.6 ± 0.0
Meath Park, SK (2012)	30.2 ± 0.0	1.3 ± 0.3	3.9 ± 0.2
Overall mean ± s.d.	31.8 ± 1.5	1.3 ± 0.2	3.4 ± 0.4

In the case of crude fat, all genotypes were impacted by the growing location and year ($p<0.01$), with the exception of Snowbird and CDC Snowdrop ($p>0.05$) (Table 4.1). CDC Fatima grown at Arborg in 2011 (0.9%, moisture-free basis) was significantly lower in crude fat than when grown at Saskatoon-Preston in 2012 (1.3%), Outlook in 2012 (1.3%) or Lacombe in 2011 (1.5%) ($p<0.01$). Taboar grown at Saskatoon-Preston in 2012 was significantly lower in crude fat (1.1%) than when grown at Lacombe in 2011 (1.4%) ($p<0.01$), yet similar in crude fat when grown at Arborg in 2011 (1.0%) or Outlook in 2012 (1.2%) ($p>0.05$). For SSNS-1, the level of crude fat in flour from seed grown at Outlook in 2012 (1.2%) or Edmonton in 2011 (1.2%) was significantly higher than when grown at Arborg in 2011 (0.9%), and significantly lower than when grown at Rosthern in 2012 (1.3%) ($p<0.01$). Flour from FB9-4 was similar in crude fat when grown at Outlook in 2012 or Edmonton in 2011 (1.3%) ($p>0.05$), but was significantly higher in crude fat than when grown at Arborg in 2011 (1.0%), and significantly lower in crude fat than when grown at Rosthern in 2012 (1.5%) ($p<0.01$). For FB18-20, the crude fat level in flour from seed grown at Arborg in 2011 (1.2%) was similar to that in seed grown at Edmonton in 2011 (1.3%) or Outlook in 2012 (1.4%) ($p>0.05$), and significantly lower than when grown at Rosthern in 2012 (1.5%) ($p<0.01$).

Environmental effects were observed for ash content for each genotype, with the exceptions of FB9-4 and FB18-20 (Table 4.1). Flours from Taboar grown at Arborg in 2011 (2.9%), Saskatoon-Preston in 2012 (3.0%) or Outlook in 2012 (3.0%) had similar ash levels ($p>0.05$), which were significantly lower than when grown at Lacombe in 2011 (3.4%) ($p<0.05$). For CDC Fatima, flours from seed grown at Arborg in 2011 (3.3%) or Saskatoon-Preston in 2012 (3.5%) had similar ash levels ($p>0.05$), which were significantly higher than when grown at Lacombe in 2011 (2.7%) and significantly lower than when grown at Outlook in 2012 (4.1%) ($p<0.01$). Flour from SSNS-1 grown at Outlook in 2012 (2.8%) was similar in ash to those prepared from seed grown at Rosthern in 2012 (3.0%) or Edmonton in 2011 (3.2%) ($p>0.05$), but significantly lower than when grown at Arborg in 2011 (3.6%) ($p<0.05$). For CDC Snowdrop, flour prepared from seed grown at Edmonton in 2011 (3.0%) was similar in ash to flour from seed grown at Rosthern in 2012 (3.6%) or Saskatoon-Sutherland in 2011 (3.7%) ($p>0.05$), but significantly lower in ash than when grown at Meath Park in 2012 (3.8%) ($p<0.05$). Flours from Snowbird grown at Lacombe in 2011 (2.8%) or Saskatoon-Sutherland in 2012 (3.0%) were

similar in ash ($p>0.05$) and significantly lower in ash than when grown at Melita in 2011 (3.7%) or Outlook in 2012 (4.3%) ($p<0.01$).

b) Faba bean protein isolates

A one-way ANOVA revealed that the effects of genotype on the protein and ash contents of protein isolates were non-significant ($p>0.05$) (Table 4.2). The average moisture (fresh weight basis), protein and ash levels in the isolates were 4.7%, 93.9% (d.b.), and 5.8% (d.b.), respectively. However, most exhibited some environmental influences related to location and year within each genotype. Crude fat was measured for only a small number of isolates during preliminary experiments, where it was found to be at negligible levels due to the defatting process. In the case of protein content, isolates prepared from FB9-4 and FB18-20 were found to be similar regardless of their growing location and year of harvest ($p>0.05$). In contrast, isolates prepared from CDC Snowdrop and SSNS-1 were found to have different protein levels at all locations/years, ranging between ~90.5% and 95.0%, and ~93.0% and ~99.4%, respectively ($p<0.01$). Isolates prepared from Snowbird grown at Saskatoon-Sutherland in 2011 (93.5%) were significantly higher in protein than when grown at Melita in 2011 (92.0%), and significantly lower than when grown at Outlook in 2012 (94.7%) or Lacombe in 2011 (94.9%) ($p<0.01$). For CDC Fatima, the protein level in the isolate prepared from seed grown at Outlook in 2012 (94.9%) was significantly lower than when grown at Arborg in 2011 (96.6%), and significantly higher than when grown at Saskatoon-Preston in 2012 (93.8%) or Lacombe in 2011 (93.9% ($p<0.01$). The level of protein in the isolate prepared from Taboar was similar when grown at Saskatoon-Preston in 2012 (94.4%), Outlook in 2012 (93.7%) or Arborg in 2011 (94.4%) ($p>0.05$), and was significantly lower than when grown at Lacombe in 2011 (95.3%) ($p<0.01$).

In the case of ash, environmental effects were observed for each genotype with the exceptions of FB18-20, Taboar and CDC Snowdrop ($p>0.05$) (Table 4.2). Isolates prepared from CDC Fatima grown at Arborg in 2011 (5.0%) or Outlook in 2012 (5.3%) had similar ash levels ($p>0.05$), which were significantly lower than when grown at Saskatoon-Preston in 2012 (5.9%) or Lacombe in 2011 (6.0%) ($p<0.01$). For FB9-4, isolates from seed grown at Arborg in 2011 or Edmonton in 2011 had similar ash levels (5.5%) ($p>0.05$), which were significantly higher than when grown at Rosthern in 2012 (5.1%), and significantly lower than when grown at Outlook in 2012 (6.0%) ($p<0.01$). Similarly for SSNS-1, isolates from seed grown at Outlook in 2012 and

Table 4.2. Percent protein and ash in faba bean protein isolates produced from seven genotypes in two years at several locations in Western Canada. Data represent the mean ± one standard deviation (n = 2).

	Protein (%, d.b.)	**Ash (%, d.b.)**
a) CDC Fatima		
Arborg, MB (2011)	96.6 ± 0.1	5.0 ± 0.1
Lacombe, AB (2011)	93.9 ± 0.0	6.0 ± 0.2
Outlook, SK (2012)	94.9 ± 0.2	5.3 ± 0.0
Saskatoon-Preston, SK (2012)	93.8 ± 0.0	5.4 ± 0.0
b) Taboar		
Arborg, MB (2011)	94.4 ± 0.2	6.0 ± 0.5
Lacombe, AB (2011)	95.3 ± 0.2	5.3 ± 0.3
Outlook, SK (2012)	93.7 ± 0.3	6.0 ± 0.3
Saskatoon-Preston, SK (2012)	94.3 ± 0.0	5.9 ± 0.2
c) SSNS-1		
Arborg, MB (2011)	94.5 ± 0.1	4.8 ± 0.3
Edmonton, AB (2011)	93.5 ± 0.1	5.9 ± 0.1
Outlook, SK (2012)	99.4 ± 0.1	5.9 ± 0.1
Rosthern, SK (2012)	93.0 ± 0.2	5.3 ± 0.2
d) FB9-4		
Arborg, MB (2011)	94.8 ± 0.2	5.5 ± 0.0
Edmonton, AB (2011)	92.8 ± 0.3	5.5 ± 0.2
Outlook, SK (2012)	93.2 ± 0.0	6.0 ± 0.1
Rosthern, SK (2012)	94.1 ± 1.2	5.1 ± 0.0
e) FB18-20		
Arborg, MB (2011)	93.1 ± 0.4	5.6 ± 0.0
Edmonton, AB (2011)	93.0 ± 0.2	6.3 ± 0.6
Outlook, SK (2012)	93.5 ± 0.4	6.2 ± 0.2
Rosthern, SK (2012)	92.7 ± 0.0	5.9 ± 0.1
f) Snowbird		
Lacombe, AB (2011)	94.9 ± 0.3	5.2 ± 0.1
Melita, MB (2011)	92.0 ± 0.1	6.9 ± 0.3
Outlook, SK(2012)	94.7 ± 0.2	6.2 ± 0.1
Saskatoon-Sutherland, SK (2012)	93.5 ± 0.3	5.8 ± 0.1
g) CDC Snowdrop		
Edmonton, AB (2011)	95.1 ± 0.1	5.8 ± 0.0
Saskatoon-Sutherland, SK (2011)	90.5 ± 0.1	6.4 ± 0.3
Rosthern, SK (2012)	93.3 ± 0.0	5.8 ± 0.1
Meath Park, SK (2012)	91.3 ± 0.0	6.7 ± 0.5
Overall mean ± s.d.	93.9 ± 1.6	5.8 ± 0.5

Edmonton in 2011 had similar ash levels (5.9%) ($p>0.05$), which were significantly higher than when grown at Arborg in 2011 (4.8%) or Rosthern in 2012 (5.3%) ($p<0.05$). Finally, isolates from Snowbird when grown at Lacombe in 2011 (5.2%) were found to have similar ash levels as when grown at Saskatoon-Sutherland in 2012 (5.8%) ($p>0.05$), and significantly lower in ash than when grown at Outlook in 2012 (6.2%) or Melita in 2011 (6.9%) ($p<0.01$).

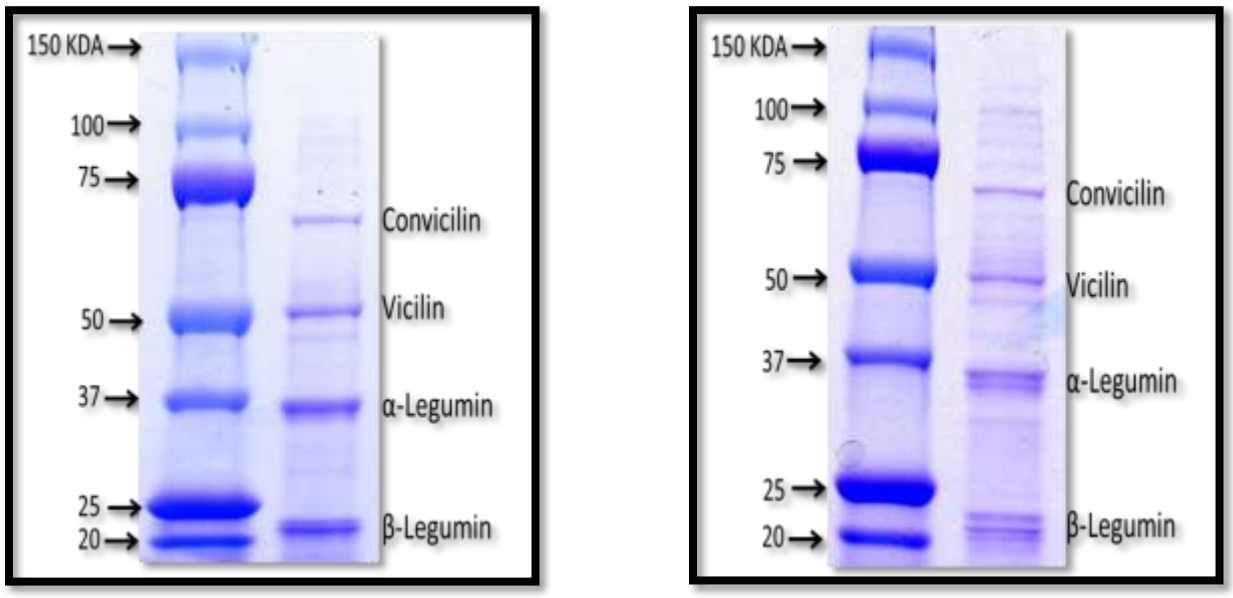
4.2 Extraction efficiency of isolate production

Faba bean protein isolates were prepared from defatted flours by an alkaline extraction process followed by isoelectric precipitation to yield isolates containing 90% (moisture free basis) or more of protein (Table 4.2). Process efficiency was described by both the isolate yield and the protein yield. The former is a measure of the amount of isolate obtained relative to the original 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 found no significant differences in isolate or protein yield due to genotype ($p>0.05$). The isolate yield across genotypes ranged between 23.5 ± 3.4% for CDC Fatima and 26.8 ± 1.7% for SSNS-1, with an average isolate yield of 25.3 ± 2.1%. Similarly, protein yield was found to range between 70.4 ± 9.7% for CDC Fatima and 79.8 ± 1.6% for Snowbird, with an average protein yield of 76.6 ± 5.4%.

4.3 Protein composition of flours and isolates

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions was used to examine the protein bands in both the flours and the protein isolates from each genotype, location and year. Using densitometry, the relative percentages of the legumin (L) [α-legumin chain (37 kDa) + β-legumin chain (20 kDa)] and vicilin (V) (50 kDa) proteins were determined and reported as the L/V ratio. Representative examples of SDS-PAGE gels for an isolate (Figure 4.1Ai) and a flour (Figure 4.1Aii) prepared from FB18-20 grown at Edmonton in 2011 are presented, along with the corresponding densitometry analysis for the isolate only (Figure 4.1B). Band identification was based on published values from Nikolic et al. (2012) and Tucci et al. (1991). For the protein isolate, four major bands were identified corresponding to molecular masses of ~73 kDa, ~51 kDa, ~34 kDa and ~21 kDa and were presumed to correspond to convicilin, vicilin, α-legumin and β-legumin chains, respectively. Similarly for the

(Ai) **(Aii)**



(B)

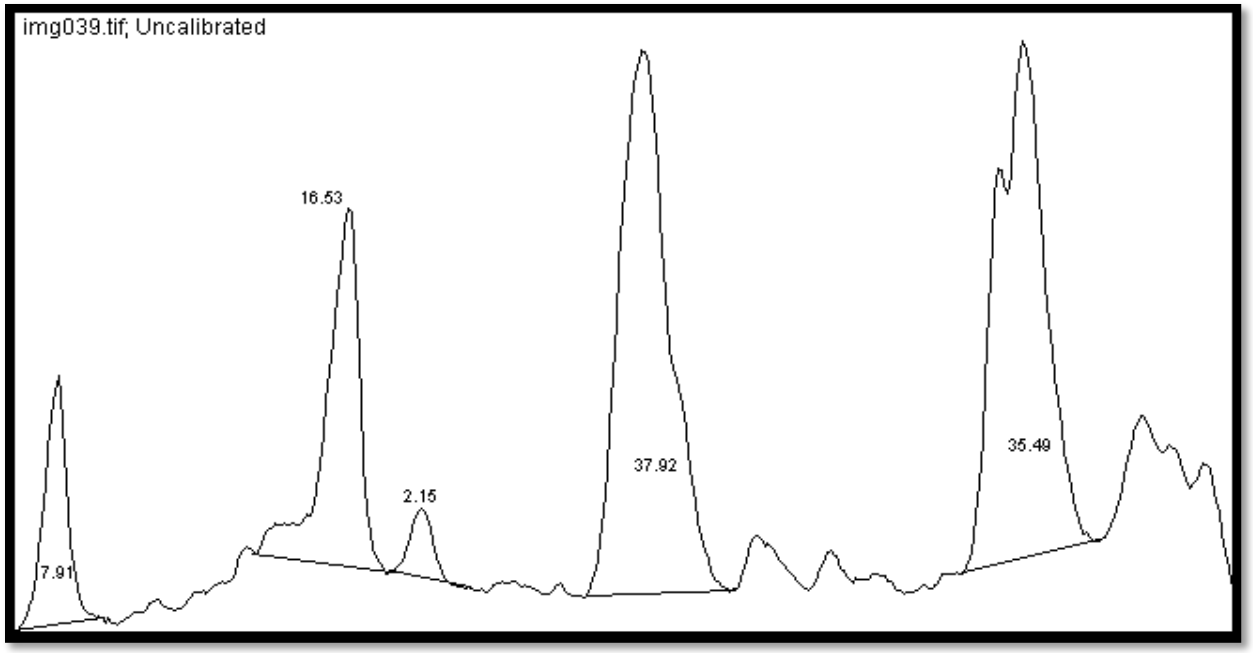


Figure 4.1 Representative examples of: A) SDS-PAGE gel for isolate (Ai) and flour (Aii) prepared from FB18-20 grown at Edmonton in 2011; and (B) a corresponding denisotmetry analysis for the isolate protein profile.

corresponding flour sample, four major bands were identified corresponding to molecular masses of ~73 kDa, ~53 kDa, ~36 kDa and ~21 kDa, again representing convicilin, vicilin, α-legumin and β-legumin chains, respectively. The molecular weight profiles for all other isolates and flour samples were very similar to those of FB18-20 grown at Edmonton in 2011. Minor (faint) bands were not included in the analysis, and might represent albumin storage proteins and/or enzymes. The extraction process used in the preparation of protein isolates from flour eliminated some bands.

The legumin:vicilin (L/V) ratio was similar in magnitude across all genotypes for both faba bean flours (range of L/V ratio 3.4-4.6; average of 3.8) and isolates (range of L/V ratio 4.0-4.9; average of 4.5) ($p>0.05$). However, growing location and year did create variability within each genotype (Figure 4.2A). In the case of isolates, only Snowbird was found to have a similar L/V ratio regardless of the location in which it was grown (Melita, Lacombe, Outlook and Saskatoon-Sutherland) ($p>0.05$). The L/V ratio determined for isolates prepared from CDC Fatima was similar in magnitude when grown at Lacombe in 2011 (L/V = 4.4), Arborg in 2011 (4.5) or Saskatoon-Preston in 2012 (4.6) ($p>0.05$), yet significantly lower than when grown at Outlook in 2012 (5.1) ($p<0.01$). Isolates prepared from Taboar had similar L/V ratios when grown at Arborg in 2011 (L/V = 3.8), Saskatoon-Preston in 2012 (4.0) or Lacombe in 2011 (4.3) ($p>0.05$), which were significantly lower than when grown at Outlook in 2012 (5.0) ($p<0.01$). The L/V ratio for isolates prepared from SSNS-1 were similar when seed was grown at Arborg in 2011 or Rosthern in 2012 (L/V = 5.1) ($p>0.05$), and significantly higher than when grown at Edmonton in 2011 (3.9) or Outlook in 2012 (4.6) ($p<0.01$). For isolates prepared from FB9-4, the L/V ratio was similar in magnitude when seed was grown at Outlook in 2012 (L/V = 5.6) or Edmonton in 2011 (5.7) ($p>0.05$), but was significantly higher than when grown at Arborg in 2011 (3.5) or Rosthern in 2012 (4.8) ($p<0.01$). For isolates prepared from FB18-20, the L/V ratio was similar when seed was grown at Rosthern in 2012 (4.2), Arborg in 2011 (4.3) or Edmonton in 2011 (4.8) ($p>0.05$), and significantly lower than when grown at Outlook in 2012 (5.8) ($p<0.01$). Finally, the L/V ratios of isolates prepared from CDC Snowdrop were similar when seed was grown at Meath Park in 2012 (3.2) or Rosthern in 2012 (3.7) ($p>0.05$), and significantly lower than when grown at Saskatoon-Sutherland in 2011 (4.8) or Edmonton in 2011 (4.5) ($p<0.01$).

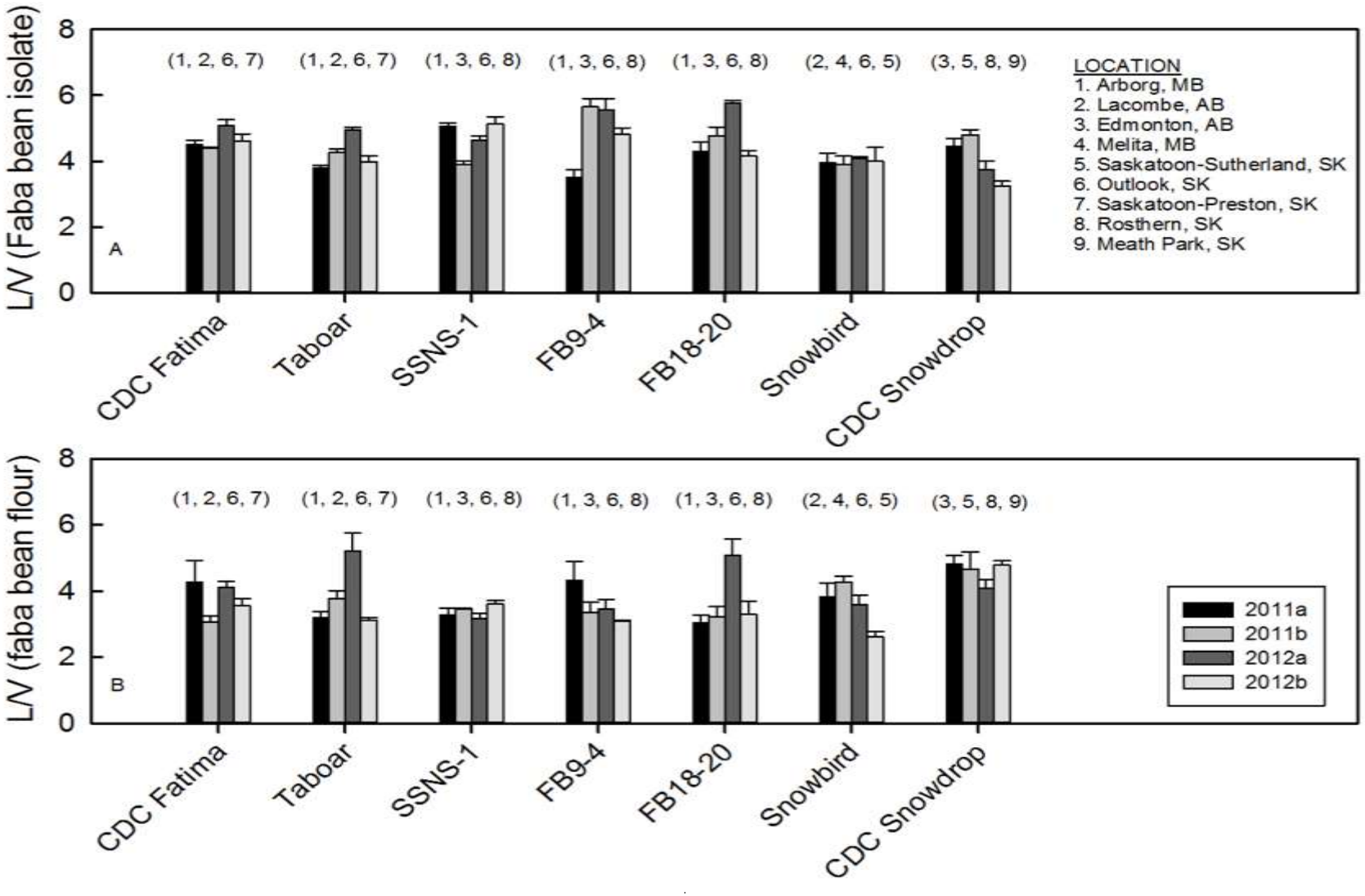


Figure 4.2 L/V ratios of faba bean isolates (A) and faba bean flours (B) as a function of genotype, location and year at pH 7.0. Data represent means ± one standard deviations (n=2).

In the case of flour samples, the mean L/V ratio for CDC Snowdrop was found to be 4.6 while for all other genotypes the mean L/V ratio was found to range between 3.4 and 3.8 (Figure 4.2B). Differences due to environment were observed for most of the genotypes, except for CDC Snowdrop whose L/V ratio was similar regardless of the year (2011, 2012) or location (Meath Park, Edmonton, Saskatoon-Sutherland, and Rosthern) (Figure 4.2B). The L/V ratios determined for flours produced from Taboar when grown at Saskatoon-Preston in 2012 (L/V = 3.1), Arborg in 2011 (3.2) or Lacombe in 2011 (3.8) were similar in magnitude ($p>0.05$), but significantly lower than when seed was grown at Outlook in 2012 (5.2) ($p<0.01$). For FB18-20, the L/V ratio of flours prepared from seed grown at Arborg in 2011 (L/V = 3.0), Edmonton in 2011 (3.2) or Rosthern in 2012 (3.3) were similar in magnitude ($p>0.05$), but significantly lower than when seed was grown at Outlook in 2012 (5.1) ($p<0.01$). CDC Fatima flour prepared from seed grown at Lacombe in 2011 had a similar L/V ratio (L/V = 3.1) as that produced from seed grown at Saskatoon-Preston in 2012 (3.5) ($p>0.05$), but lower than that for flour from seed grown at Outlook in 2012 (4.1) or Arborg in 2011 (4.2) ($p<0.01$). For SSNS-1, the L/V ratio of flour prepared from seed grown at Outlook in 2012 (L/V = 3.2) was similar to that of flour from seed grown at Arborg in 2011 (3.3) or Edmonton in 2011 (3.5) ($p>0.05$), but lower than that for flour from seed grown at Rosthern in 2012 (3.6) ($p<0.01$). For FB9-4, the L/V ratio of flour was similar when seed was grown at Rosthern in 2012 (L/V = 3.1), Edmonton in 2011 (3.3) or Outlook in 2012 (3.5) ($p>0.05$), but was significantly lower than for flour from seed grown at Arborg in 2011 (4.3) ($p<0.01$). For flours from Snowbird, the L/V ratio was similar for seed grown at Outlook in 2012 (L/V = 3.6), Lacombe in 2011 (3.8) or Melita in 2011 (4.3) ($p>0.05$), but higher than for flour from seed grown at Saskatoon-Sutherland in 2012 (2.6) ($p<0.01$).

4.4 Colour of flours and isolates

Hunter lab colour parameters (L*, a* and b*) were evaluated for faba bean flours and isolate samples as shown in Tables 4.3A and 4.3B, respectively. In this study, only genotypic differences in colour were taken in account. The overall mean of flour L*, a* and b* values were found to be 89.0, 0.4 and 16.4 respectively. The L* value for flour samples was found to be significantly different across genotypes, but varying just between ~88.3 (CDC Snowdrop, SSNS-1 and Snowbird) and ~89.5 (Taboar, CDC Fatima, FB9-4 and FB18-20) ($p<0.01$) (Table 4.3A). The b* values also varied significantly among the genotypes, where flour from CDC Snowdrop

Table 4.3A. L*, a* and b* values of faba bean flours prepared from seven genotypes grown in 2011 and 2012 at several locations in Western Canada. Values represent the mean ± one standard deviation (n = 3).

	L*	a*	b*
a) CDC Fatima			
Arborg, MB (2011)	88.9 ± 0.0	0.4 ± 0.0	15.7 ± 0.0
Lacombe, AB (2011)	88.6 ± 0.0	0.4 ± 0.0	15.8 ± 0.0
Outlook, SK (2012)	89.8 ± 0.1	0.4 ± 0.0	15.3 ± 0.0
Saskatoon-Preston, SK (2012)	89.7 ± 0.0	0.4 ± 0.0	15.9 ± 0.0
b) Taboar			
Arborg, MB (2011)	89.3 ± 0.0	0.4 ± 0.0	15.2 ± 0.0
Lacombe, AB (2011)	89.0 ± 0.0	0.4 ± 0.0	16.0 ± 0.0
Outlook, SK (2012)	89.5 ± 0.0	0.4 ± 0.0	15.1 ± 0.1
Saskatoon-Preston, SK (2012)	89.1 ± 0.0	0.4 ± 0.0	16.4 ± 0.0
c) SSNS-1			
Arborg, MB (2011)	88.8 ± 0.0	0.6 ± 0.0	16.4 ± 0.0
Edmonton, AB (2011)	88.0 ± 0.1	-0.0 ± 0.0	17.9 ± 0.0
Outlook, SK (2012)	88.7 ± 0.1	0.5 ± 0.1	18.1 ± 0.1
Rosthern, SK (2012)	89.1 ± 0.0	0.4 ± 0.0	17.2 ± 0.0
d) FB9-4			
Arborg, MB (2011)	89.0 ± 0.1	0.6 ± 0.0	14.7 ± 0.0
Edmonton, AB (2011)	89.5 ± 0.1	0.6 ± 0.0	14.8 ± 0.0
Outlook, SK (2012)	89.6 ± 0.0	0.4 ± 0.0	15.5 ± 0.0
Rosthern, SK (2012)	89.5 ± 0.0	0.6 ± 0.0	15.6 ± 0.0
e) FB18-20			
Arborg, MB (2011)	89.2 ± 0.0	0.4 ± 0.0	15.4 ± 0.0
Edmonton, AB (2011)	89.4 ± 0.2	0.5 ± 0.0	15.1 ± 0.0
Outlook, SK (2012)	89.8 ± 0.1	0.5 ± 0.0	15.4 ± 0.0
Rosthern, SK (2012)	89.3 ± 0.0	0.4 ± 0.0	16.1 ± 0.1
f) Snowbird			
Lacombe, AB (2011)	88.5 ± 0.0	0.2 ± 0.0	15.9 ± 0.0
Melita, MB (2011)	88.6 ± 0.0	0.1 ± 0.0	16.8 ± 0.1
Outlook, SK(2012)	88.9 ± 0.0	0.3 ± 0.0	17.6 ± 0.0
Saskatoon-Sutherland, SK (2012)	89.0 ± 0.1	0.3 ± 0.0	17.7 ± 0.0
g) CDC Snowdrop			
Edmonton, AB (2011)	87.7 ± 0.1	-0.2 ± 0.0	18.4 ± 0.0
Saskatoon-Sutherland, SK (2011)	88.6 ± 0.0	0.5 ± 0.0	18.4 ± 0.0
Rosthern, SK (2012)	88.7 ± 0.0	0.3 ± 0.0	17.6 ± 0.0
Meath Park, SK (2012)	88.1 ± 0.1	0.3 ± 0.0	19.3 ± 0.0
Overall mean ± s.d	89.0 ± 0.5	0.4 ± 0.2	16.4 ± 1.3

was more yellowish in colour (b* = 18.4) as compared to other genotypes (b* = ~15 to ~17) ($p<0.01$). Finally, differences in a* values for flour samples were found to be not significant among genotypes, although Snowbird and CDC Snowdrop flours (a* = ~0.2) were observed to be slightly less reddish in colour as compared to the other genotypes (a* = ~0.3 to ~0.6).

The L*, a* and b* values for faba bean protein isolates were found to be similar among genotypes ($p>0.05$), with overall means of 72.0, 1.8 and 20.3, respectively (Table 4.3B). The isolate produced from Snowbird was darker in colour (L* = 68.6) compared to Taboar (L* = 73.4), which was similar in brightness to all other genotypes (L* = ~71 to 73). Values for a* varied between 1.4 and 2.1, whereas b* values varied between 18.3 and 21.6. Again, similar to flour colours, the isolates prepared from Snowbird and CDC Snowdrop were less reddish in colour (a* = ~1.4 to 1.7) as compared to all other genotypes (a* = ~1.9 to ~2.1). Moreover, Snowbird was darker and more bluish in colour than the other genotypes. Taboar yielded the brightest faba bean protein isolate (L* = 73.4), and CDC Fatima and CDC Snowdrop the most reddish (a* = 2.1) and yellowish (b* = 21.6).

4.5 Physicochemical properties of the protein isolates

The physicochemical properties (i.e., surface charge, hydrophobicity, and interfacial and surface tension) of faba bean isolates were tested at pH 7.0 as a function of genotype, location and year of harvest. However, due to limited seed availability, the locations differed among genotypes and years. Therefore, statistical analysis could consider only the impact of genotype as a whole. However, environmental effects were discussed in relation to each genotype separately.

4.5.1 Zeta potential

Zeta potential (ZP) (surface charge) is a measure of the net electric charge surrounding a protein, the magnitude of which is related to the relative stability of the protein in solution. For instance, if the zeta potential is high (~ +/- 30 mV), particles tend to repel one another in solution due to electrostatic repulsive forces, whereas if the magnitude of ZP is less than approximately +/- 30 mV, instability occurs and proteins tend to aggregate more since repulsive charges are lower. In the present study, differences in zeta potential among protein isolates were non-significant across all genotypes, with an average potential of + 22.1 mV at pH 7.0 ($p>0.05$) (Figure 4.3A). Moreover, no significant influence of environmental factors was observed as

a function of location or year of harvest within any of the genotypes ($p>0.05$) (Figure 4.3A).

Table 4.3B. L*, a* and b* values of faba bean protein isolates prepared from seven genotypes grown in 2011 and 2012 at several locations in Western Canada. Values represent the mean ± one standard deviation (n = 3).

.

	L*	a*	b*
a) CDC Fatima			
Arborg, MB (2011)	71.4 ± 0.1	2.1 ± 0.0	19.6 ± 0.1
Lacombe, AB (2011)	71.3 ± 0.1	1.8 ± 0.0	18.6 ± 0.1
Outlook, SK (2012)	74.6 ± 0.1	2.6 ± 0.0	22.1 ± 0.1
Saskatoon-Preston, SK (2012)	70.5 ± 0.1	2.0 ± 0.0	18.9 ± 0.1
b) Taboar			
Arborg, MB (2011)	73.7 ± 0.1	2.2 ± 0.0	21.2 ± 0.1
Lacombe, AB (2011)	71.9 ± 0.0	1.6 ± 0.0	17.9 ± 0.0
Outlook, SK (2012)	75.0 ± 0.0	1.1 ± 0.0	19.4 ± 0.1
Saskatoon-Preston, SK (2012)	73.0 ± 0.2	2.7 ± 0.0	23.2 ± 0.0
c) SSNS-1			
Arborg, MB (2011)	73.8 ± 0.0	2.0 ± 0.0	21.4 ± 0.0
Edmonton, AB (2011)	70.9 ± 0.2	2.3 ± 0.0	21.4 ± 0.1
Outlook, SK (2012)	73.9 ± 0.1	1.9 ± 0.0	22.6 ± 0.1
Rosthern, SK (2012)	72.2 ± 0.1	1.6 ± 0.0	19.9 ± 0.1
d) FB9-4			
Arborg, MB (2011)	71.9 ± 0.2	1.7 ± 0.0	19.6 ± 0.0
Edmonton, AB (2011)	73.5 ± 0.1	2.2 ± 0.0	21.1 ± 0.1
Outlook, SK (2012)	75.5 ± 0.0	1.8 ± 0.0	21.7 ± 0.0
Rosthern, SK (2012)	71.1 ± 0.1	1.9 ± 0.0	18.5 ± 0.0
e) FB18-20			
Arborg, MB (2011)	71.3 ± 0.2	1.9 ± 0.0	19.8 ± 0.1
Edmonton, AB (2011)	74.6 ± 0.2	1.9 ± 0.0	21.2 ± 0.1
Outlook, SK (2012)	71.1 ± 0.1	1.8 ± 0.0	18.3 ± 0.1
Rosthern, SK (2012)	73.0 ± 0.1	1.9 ± 0.0	21.2 ± 0.0
f) Snowbird			
Lacombe, AB (2011)	66.5 ± 0.1	1.5 ± 0.0	16.0 ± 0.1
Melita, MB (2011)	65.3 ± 0.2	0.8 ± 0.0	14.8 ± 0.0
Outlook, SK(2012)	71.9 ± 0.1	1.7 ± 0.0	21.6 ± 0.0
Saskatoon-Sutherland, SK (2012)	70.7 ± 0.2	1.6 ± 0.0	20.9 ± 0.1
g) CDC Snowdrop			
Edmonton, AB (2011)	72.4 ± 0.1	1.8 ± 0.0	23.4 ± 0.0
Saskatoon-Sutherland, SK (2011)	71.2 ± 0.1	1.9 ± 0.0	20.9 ± 0.1
Rosthern, SK (2012)	73.4 ± 0.1	1.7 ± 0.0	21.6 ± 0.0
Meath Park, SK (2012)	69.8 ± 0.1	1.6 ± 0.0	20.7 ± 0.1
Overall mean ± s.d	72.0 ± 2.3	1.8 ± 0.4	20.3 ± 2.0

4.5.2 Surface hydrophobicity

The surface hydrophobicity (SH) of faba bean protein isolates was estimated by measuring intrinsic fluorescence values using a spectroflourometer. Fluorescent intensity arises from the exposure of aromatic amino acid residues (e.g., tryptophan, tyrosine and phenylalanine) in the solvent. Proteins with higher hydrophobicity have higher intrinsic fluorescence. Overall, intrinsic fluorescence values for faba bean protein isolates were found to be similar in magnitude across all genotypes ($p>0.05$). However, differences due to environment were observed for some genotypes because of the influence of location or year of harvest (Figure 4.3B). The maximum fluorescent intensity ranged between 45.4 arbitrary units (a.u.) and 51 a.u. across genotypes, with an overall mean of 47.2 a.u.. Moreover, the intrinsic fluorescence for Taboar was similar regardless of the year (2011, 2012) or location (Arborg, Edmonton, Outlook and Rosthern), and so too for FB9-4 (Arborg, Edmonton, Outlook and Rosthern) and CDC Snowdrop (Meath Park, Edmonton, Saskatoon-Sutherland and Rosthern) ($p>0.05$). Similar intrinsic fluorescence values were observed for SSNS-1 when grown at Outlook in 2012 (SH = 47.3 a.u.), Rosthern in 2012 (48.5 a.u.) or Arborg in 2011 (50.0 a.u.) ($p>0.05$), and which were significantly higher than when grown at Edmonton in 2011 (43.3 a.u.) ($p<0.05$). For FB18-20 isolates, intrinsic fluorescence values were similar in magnitude when seed was grown at Outlook in 2012 (SH = 50.6 a.u.), Rosthern in 2012 (50.3 a.u.) or Arborg in 2011 (50.0 a.u.) ($p>0.05$), and which were significantly higher than when grown at Edmonton in 2011 (37.0 a.u.) ($p<0.05$). Faba bean isolates prepared from Snowbird when grown at Outlook in 2012 (SH = 50.0 a.u.) showed significantly higher intrinsic fluorescence values than when grown at Saskatoon-Sutherland in 2012 (44.4 a.u.), Melita in 2011 (45.5 a.u.) or Lacombe in 2011 (46.0 a.u.) ($p<0.05$). Intrinsic fluorescence values for isolates from CDC Fatima were similar in magnitude when seed was grown at Saskatoon-Preston in 2012 (SH = 50.7 a.u.) or Arborg in 2011 (46.8 a.u.) ($p>0.05$), but significantly higher than when grown at Outlook in 2012 (44.5 a.u.) or Lacombe in 2011 in (45.2 a.u.) ($p<0.05$).

4.5.3 Surface and interfacial tension

Proteins can be used as emulsifiers to form stable emulsions and foams. Proteins act by migrating to the oil-water or air-water interface, unravel and re-orient such that hydrophobic moieties are facing toward the lipid or air phase, and the hydrophilic moieties are oriented toward the polar aqueous phase (Walstra, 2003). The proteins then interact to form a viscoelastic

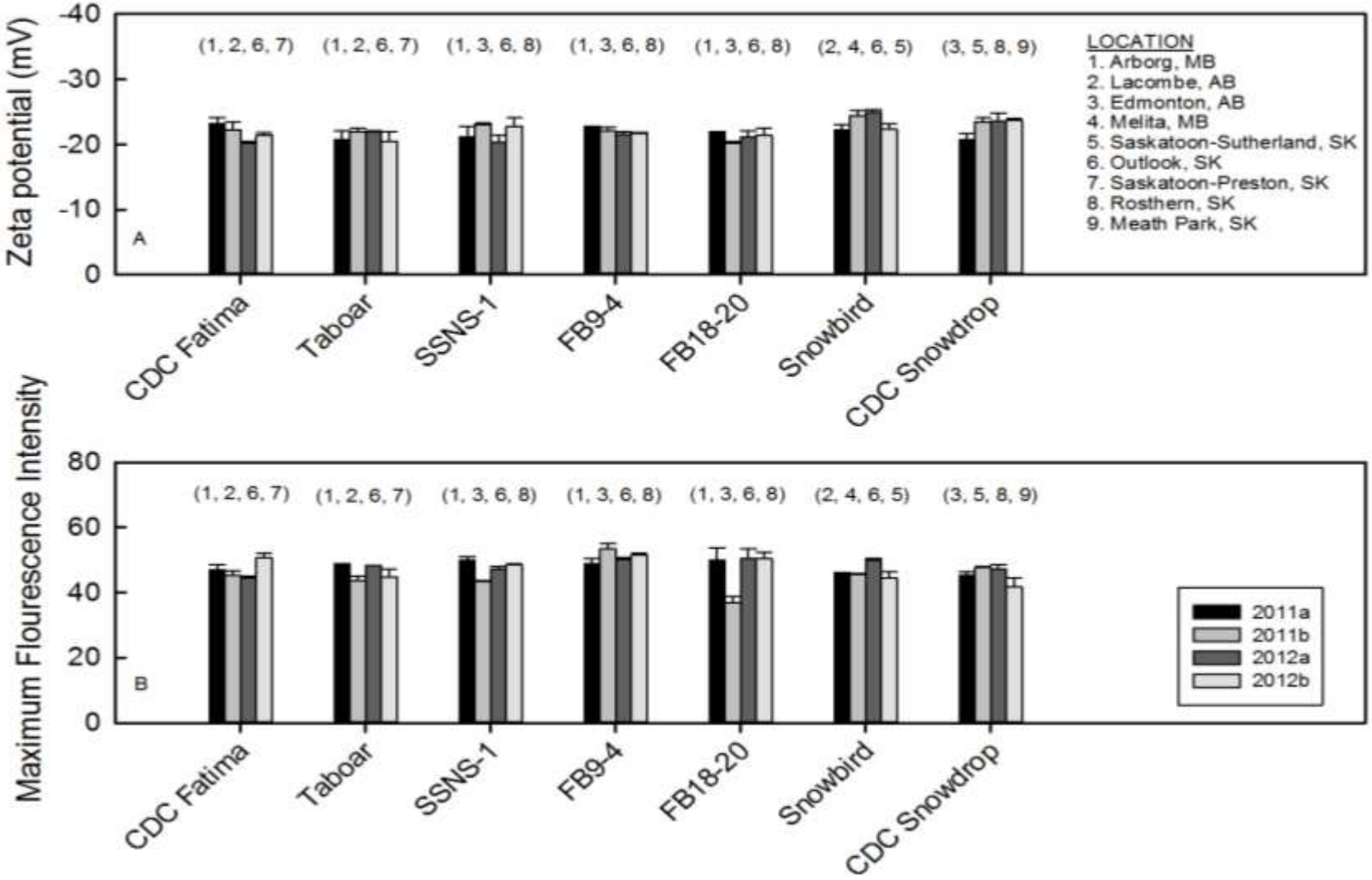


Figure 4.3 Surface charge (zeta potential) (A) and intrinsic florescence (B) of faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n = 2).

film surrounding a lipid droplet or gas bubble (Tcholakova et al., 2006). The process acts to lower the surface tension (ST) between the two phases to produce smaller, more stable droplets of the dispersed phase. In this study, the surface tension at the air-water interface without protein was found to be 95.3 mN/m. The addition of protein would be expected to reduce this tension. The surface tension at the air-protein solution interface was found to be similar across all genotypes (~65 mN/m) ($p>0.05$). However, some differences were observed due to the effect of location or year of harvest with some genotypes (Figure 4.4A). A one-way ANOVA revealed that the effect of genotype on surface tension was not significant ($p>0.05$). With the exception of Taboar, all genotypes had similar surface tension values irrespective of the year or location of harvest ($p>0.05$). The surface tension for isolates prepared from Taboar grown at Arborg in 2011 (ST = 63.6 mN/m) was similar in magnitude as when grown at Saskatoon-Preston in 2012 (65 mN/m) or Lacombe in 2011 (66 mN/m) ($p>0.05$), but significantly lower than when grown at Outlook in 2012 (68 mN/m).

Interfacial tension (IT) also was found to be similar irrespective of genotype ($p>0.05$); however, some influence due to of location or year was noticed among some genotypes (Figure 4.4B). The interfacial tension ranged between 10.4 mN/m and 11.0 mN/m, with an overall mean value of 10.7 mN/m. Interfacial tension at the oil-water interface in the absence of protein was 26.2 mN/M. Interfacial tension for FB18-20 was found to be similar irrespective of year (2011, 2012) or location (Arborg, Edmonton, Outlook and Rosthern), and so too for CDC Fatima (Arborg, Lacombe, Outlook, and Saskatoon-Preston), Taboar (Arborg, Lacombe, Outlook, and Saskatoon-Preston) and Snowbird (Melita, Lacombe, Outlook, and Saskatoon-Sutherland) ($p>0.05$). Interfacial tension for isolates prepared from FB9-4 was found to be similar in magnitude when seed was grown at Arborg in 2011 (IT = 10.2 mN/m), Rosthern in 2012 (10.2 mN/m) or Outlook in 2012 (10.5 mN/m) ($p>0.05$), and was significantly lower than when grown at Edmonton in 2011 (11.4 mN/m) ($p<0.05$). SSNS-1 grown at Arborg in 2011 (IT = 10.5 mN/m) exhibited similar interfacial tension values for faba bean isolates when grown at Rosthern in 2012 (9.9 mN/m) or at Outlook in 2012 (10.7 mN/m) ($p>0.05$), but significantly lower than when grown at Edmonton in 2011 (11.2 mN/m) ($p<0.05$). The interfacial tension for isolates prepared from CDC Snowdrop was similar when seed was grown at Edmonton in 2011 (IT = 11.4 mN/m) and Saskatoon-Sutherland in 2011 (11.0 mN/m) ($p>0.05$), but significantly higher than when grown at MeathPark in 2012 (10.7 mN/m) or Rosthern in 2012 (10.0 mN/m) ($p<0.05$).

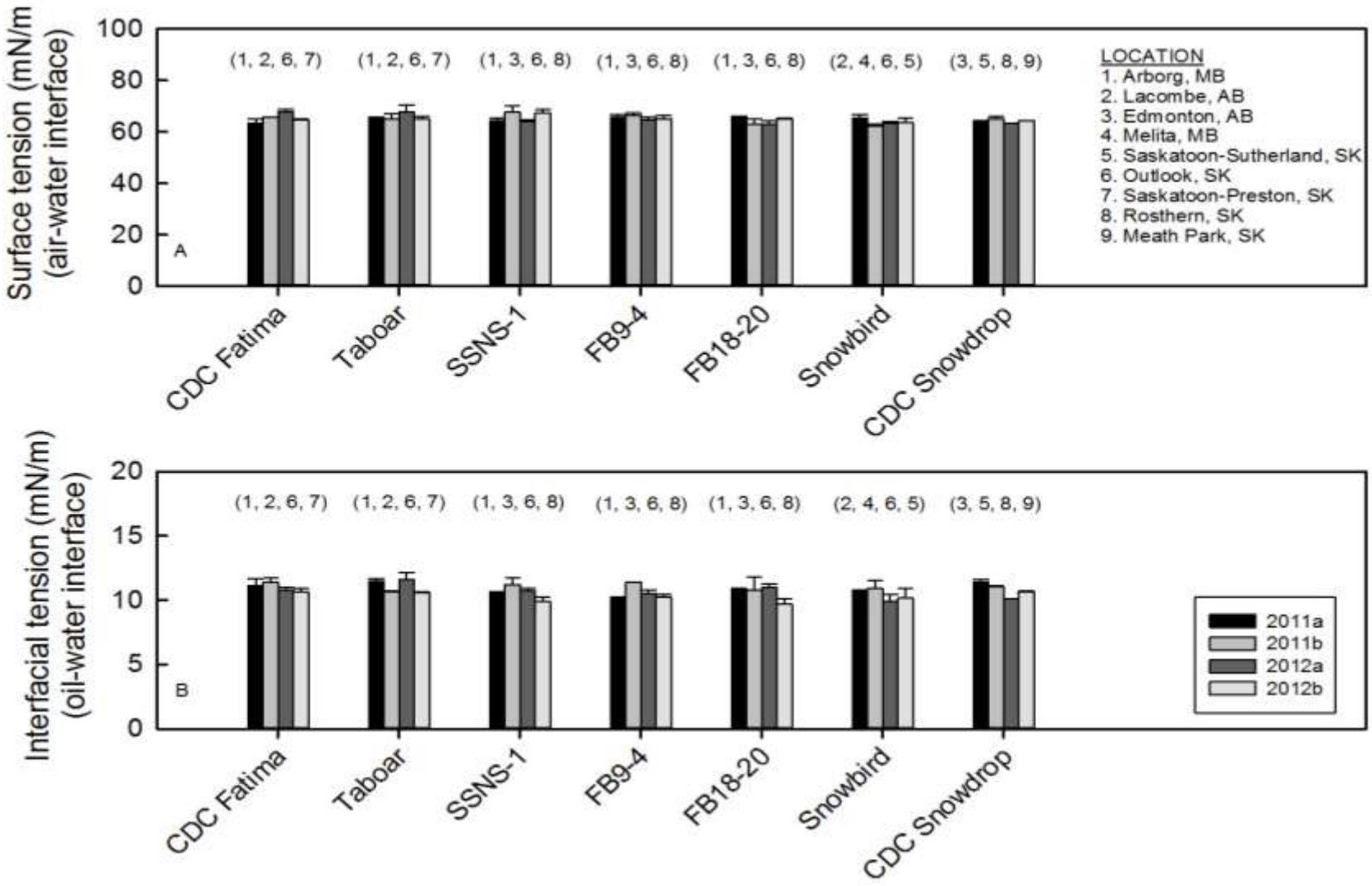


Figure 4.4. Surface (A) and interfacial (B) tension of faba bean protein isolates at the air-water and oil-water interface, respectively, as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n = 2).

4.6 Functional properties of the protein isolates

4.6.1 Oil holding capacity

Oil holding capacity (OHC) is a measure of the amount of oil absorbed per gram of protein material. OHC of proteins in foods is dependent on factors such as the protein content, the distribution of hydrophobic amino acids, droplet size and the oil type (Pearce and Kinsella, 1978). The OHC for isolates prepared from faba bean was similar in magnitude across genotypes (average = 5.7 g/g) ($p>0.05$) (Figure 4.5). However, some environmental effects were observed in response to location or year of harvest within a genotype. OHC values for FB9-4 were similar irrespective of year (2011, 2012) or location (Arborg, Edmonton, Outlook and Rosthern), and so too for Snowbird (Melita, Lacombe, Outlook and Saskatoon-Sutherland) ($p>0.05$). For CDC Fatima, OHC values were similar when seed was grown at Saskatoon-Preston in 2012 (OHC = 5.0 g/g) or Lacombe in 2011 (5.3 g/g) ($p>0.05$), and significantly lower than when grown at Outlook in 2012 (6.3 g/g) and Arborg in 2011 (6.3 g/g) ($p<0.01$). Similar OHC values were observed for Taboar when grown at Arborg in 2011 (OHC = 4.9 g/g) or Outlook in 2012 (5.2 g/g) ($p>0.05$), which were significantly lower than when grown at Saskatoon-Preston in 2012 (6.4 g/g) or Lacombe in 2011 (7.0 g/g) ($p<0.01$). OHC values for FB18-20 were similar in magnitude when seed was grown at Arborg in 2011 (OHC = 6.2 g/g), Outlook in 2012 (6.3 g/g) or Edmonton in 2011 (6.4 g/g) ($p>0.05$), and significantly higher than when grown at Rosthern in 2012 (4.5 g/g) ($p<0.01$). The OHC of SSNS-1 was similar in magnitude when seed was grown at Rosthern in 2012 (OHC = 4.6 g/g) or Edmonton in 2011 (5.4 g/g) ($p>0.05$), and significantly lower than when grown at Arborg in 2012 (6.8 g/g) or Outlook in 2012 (6.6 g/g) ($p<0.01$). The OHC of CDC Snowdrop when grown at Edmonton in 2011 (OHC = 6.4 g/g) was significantly higher than when grown at Meath Park in 2012 (5.1 g/g), Saskatoon-Sutherland in 2011 (5.1 g/g) or Rosthern in 2011 (4.9 g/g) ($p<0.05$).

4.6.2 Emulsion capacity and creaming stability

Emulsion capacity (EC) refers to the amount of oil homogenized per gram of protein before reaching an inversion point where the oil-in-water emulsion turns into a water-in-oil emulsion (Pearce and Kinsella, 1978). Creaming stability (CS) refers to the ability of a protein-stabilized emulsion to resist creaming, which occurs due to the density difference between oil and water phase where the oil molecules accumulate at the top of the emulsion due to their low

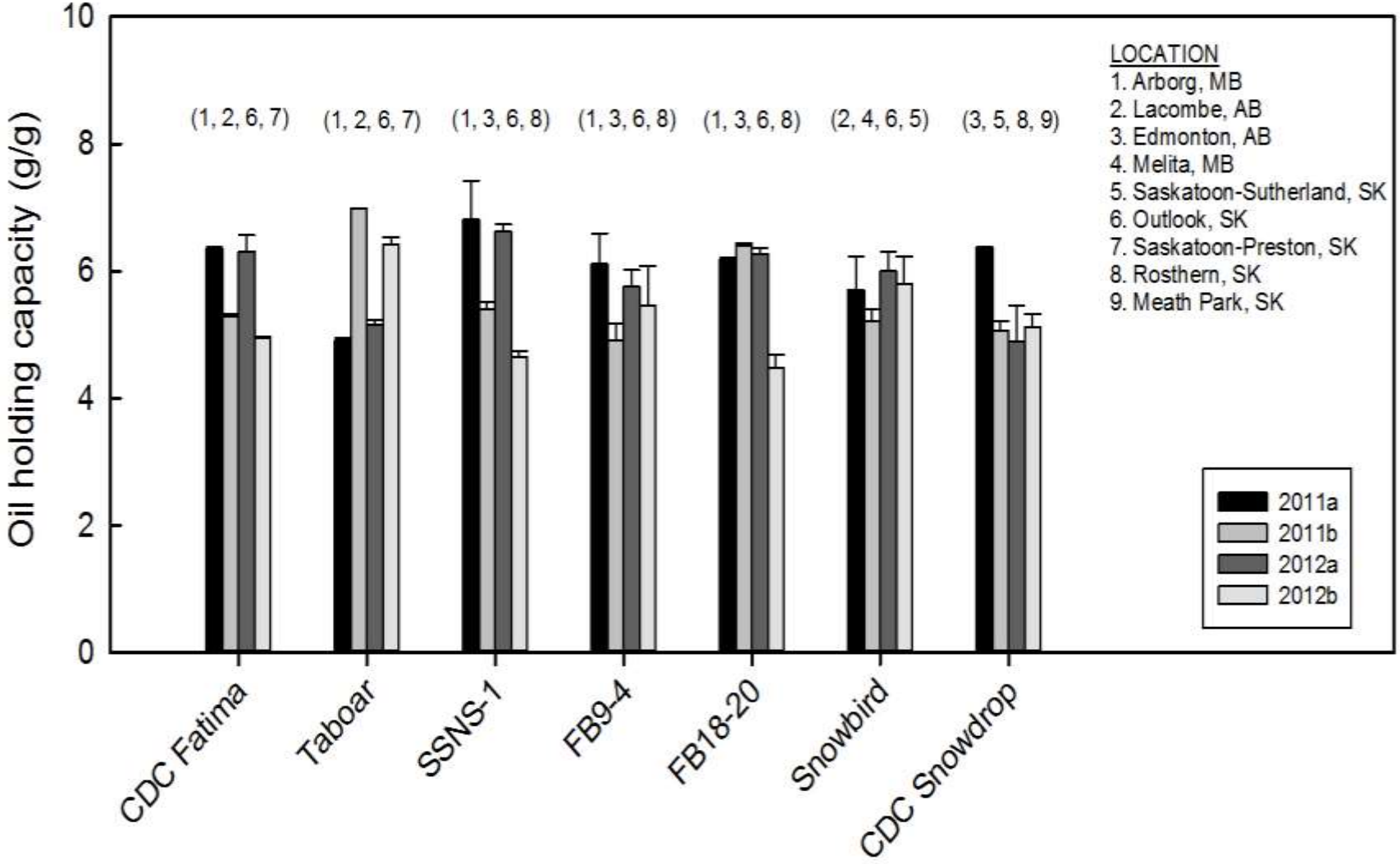


Figure 4.5. Oil holding capacity (g/g) of faba bean protein isolates as a function of genotype, location and year. Data represent the mean ± one standard deviation (n=2).

density compared to water molecules (McClements, 2007). Emulsion capacity values were similar in magnitude across genotypes (average = 184 g/g) ($p>0.05$), and only in a few cases differences due to environment were observed (Figure 4.6). The EC for isolates prepared from CDC Snowdrop was found to be similar irrespective of the year (2011, 2012) or location (Meath Park, Edmonton, Saskatoon-Sutherland and Rosthern), and so too for CDC Fatima (Arborg, Lacombe, Outlook and Saskatoon-Preston), Taboar (Arborg, Lacombe, Outlook and Saskatoon-Preston), SSNS-1 (Arborg, Edmonton, Outlook and Rosthern) and FB9-4 (Arborg, Edmonton, Outlook and Rosthern) ($p>0.05$). Similar EC values were observed for isolates prepared from FB18-20 when grown at Arborg in 2011 (EC = 169 g oil/g protein) or Edmonton in 2011 (181 g oil/g protein) ($p >0.05$), but values were significantly lower than when seed was grown at Outlook in 2012 (200 g oil/g protein) or Rosthern in 2012 (194 g oil/g protein) ($p<0.05$). For isolates prepared from Snowbird, similar EC values were observed when seed was grown at Lacombe in 2011 (EC = 169 g oil/g protein) and Saskatoon-Sutherland in 2011 (181 g oil/g protein) ($p>0.05$), and values were significantly lower than when seed was grown at Melita in 2011 (200 g oil/g protein) or Outlook in 2012 (194 g oil/g protein) ($p<0.05$). Similar to EC, CS values were similar overall (average = 94%) ($p>0.05$), and with the exception of CDC Snowdrop, no significant differences were observed due to location or year of harvest (Figure 4.7). The CS values for isolates prepared from CDC Snowdrop when grown at Edmonton in 2011 or Saskatoon-Sutherland in 2011 were similar (CS = 94%) ($p>0.05$), and significantly lower than when seed was grown at Meath Park in 2012 (95%) or Rosthern in 2012 (97%) ($p<0.05$).

4.6.3 Emulsion activity and stability indices

The emulsification activity index (EAI) refers to the ability of a protein to form an emulsion and is a measure of the interfacial area stabilized per unit (gram) of protein, whereas the emulsification stability index (ESI) is a measure of the stability of the same emulsion to resist any changes over a defined period of time (Boye et al., 2010b). For all of the genotypes tested, values for both EAI (average = 13 m^2/g) and ESI (average = 10.7 min) were similar irrespective of genotype, location or year of harvest (2011, 2012) ($p>0.05$) (Figure 4.8).

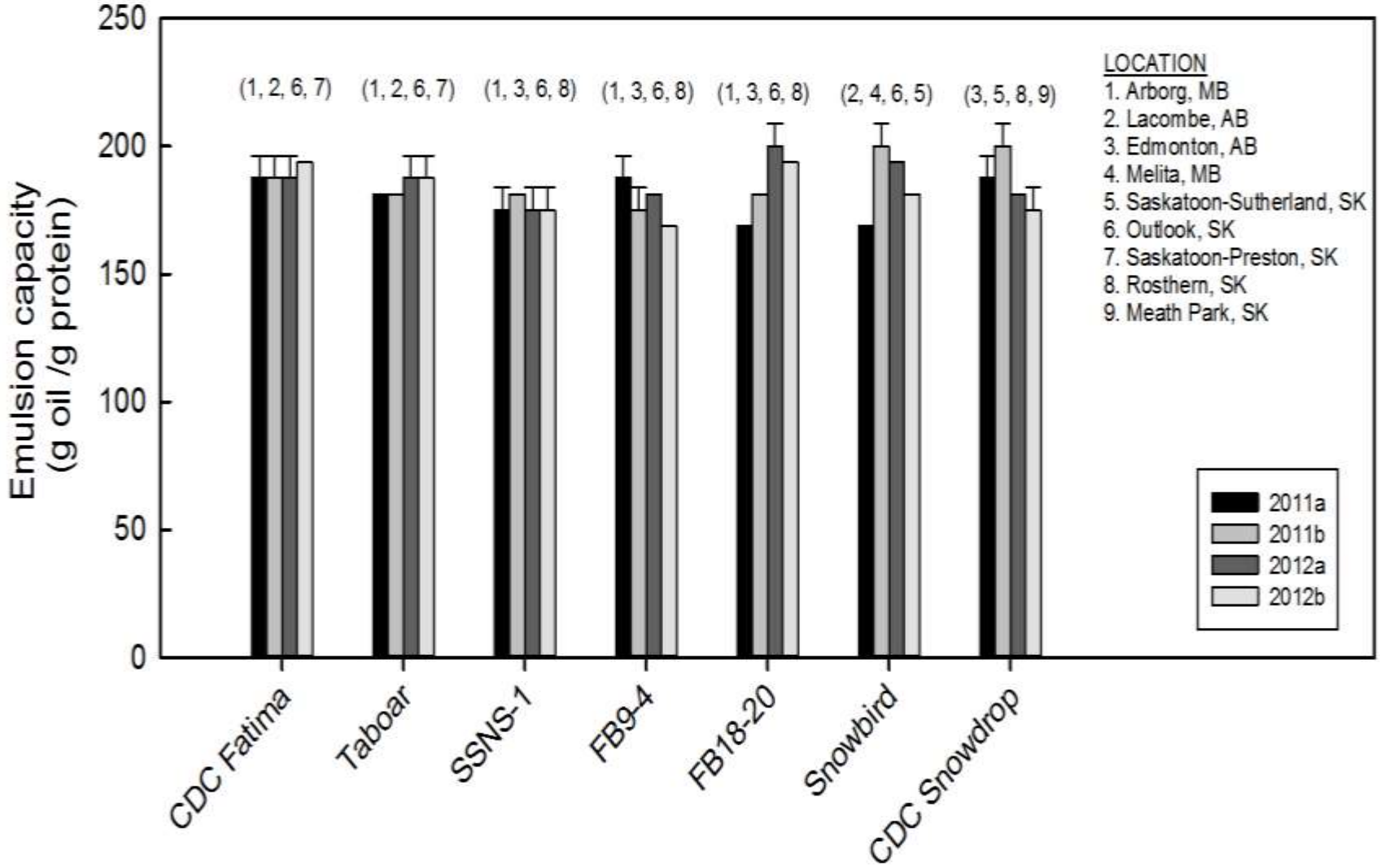


Figure 4.6. Emulsion capacity of faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n = 2).

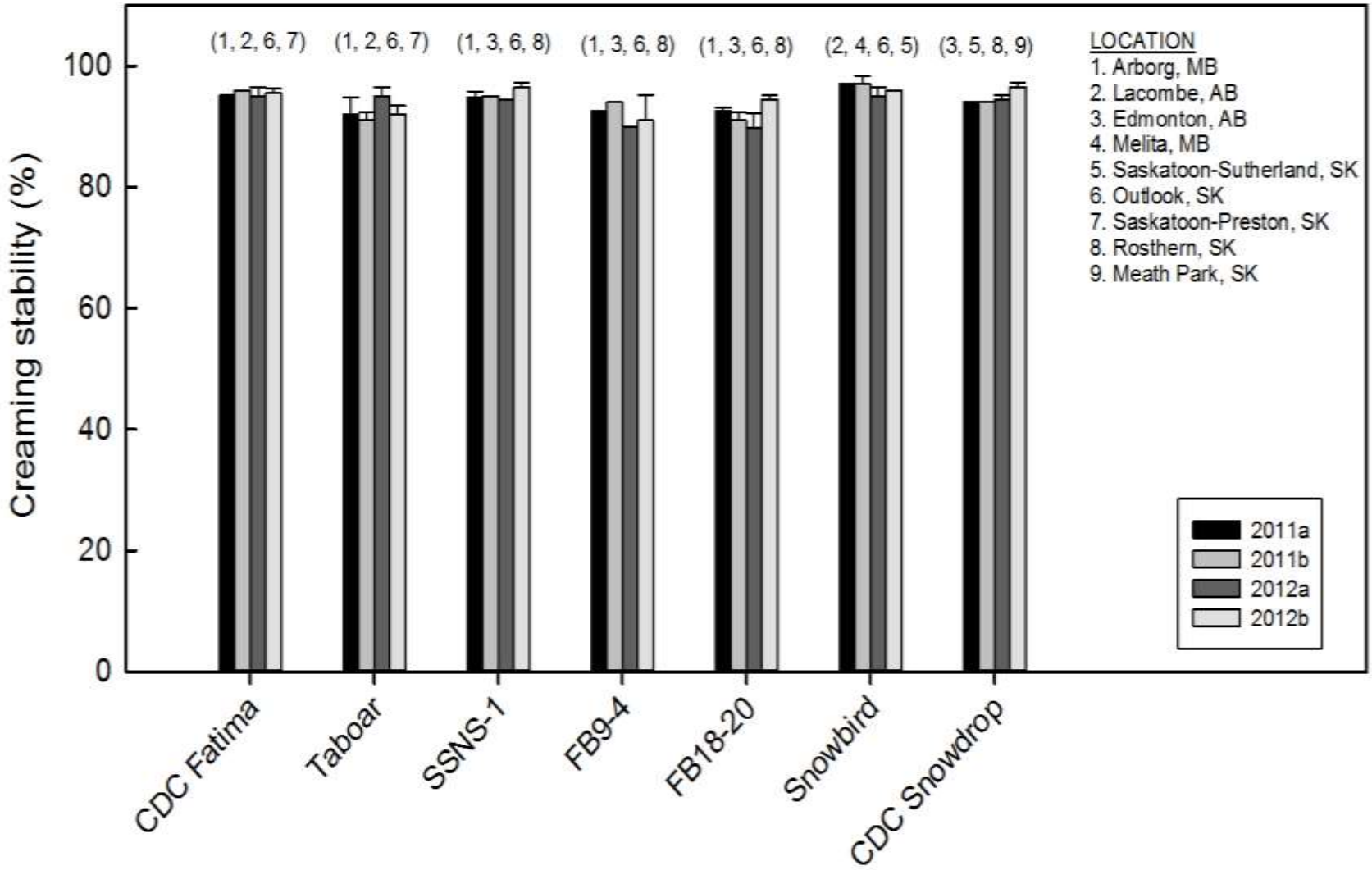


Figure 4.7. Creaming stability (%) of faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n=2).

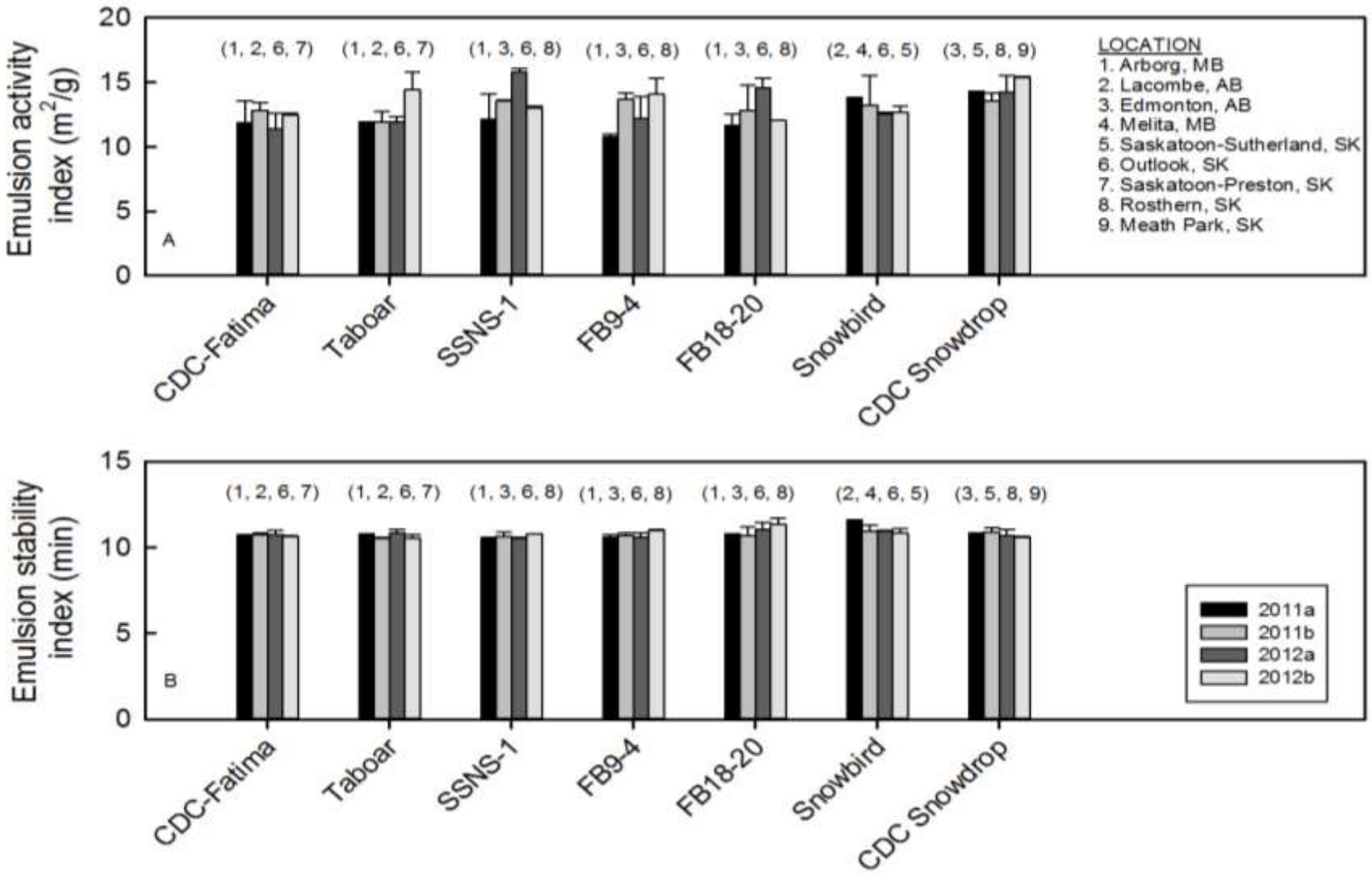


Figure 4.8. Emulsion activity (A) and stability (B) indices for faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n = 2).

4.6.4 Foaming capacity and stability

Foaming capacity (FC) refers to the volume of foam generated under a given set of homogenization and solvent conditions, whereas foam stability (FS) is related to the ability of that same foam to resist foam degradation due to the drainage of solvent from the foam lamellae. Foaming capacity values for the faba bean protein isolates were similar in magnitude irrespective of genotype (average = 162%) ($p>0.05$); however, some environmental effects were observed in response to location or year of harvest within a genotype (Figure 4.9A). Values for CDC Fatima were similar irrespective of the year (2011, 2012) or location (Arborg, Lacombe, Outlook and Saskatoon-Preston), and so too for FB9-4 (Arborg, Edmonton, Outlook and Rosthern), Taboar (Arborg, Lacombe, Outlook and Saskatoon-Preston) and SSNS-1 (Arborg, Edmonton, Outlook and Rosthern) ($p>0.05$). In contrast, isolates prepared from FB18-20 were found to have similar FC values when seed was grown at Arborg in 2011, Edmonton in 2011 or Outlook in 2012 (FC = ~162%) ($p>0.05$), and lower values than when grown at Rosthern in 2012 (183%) ($p<0.01$). Similar FC values were observed for isolates from Snowbird grown at Lacombe in 2011 or at Outlook in 2012 (FC = 150%) ($p>0.05$), which were significantly lower than when seed was grown at Melita in 2012 (175%) or Saskatoon-Sutherland in 2011 (163%) ($p<0.05$). FC values of isolates prepared from CDC Snowdrop when grown at Edmonton in 2011 (FC = 153%) were found to be similar to values for isolates from seed grown at Saskatoon-Sutherland in 2011 (163%) or Rosthern in 2012 (168%) ($p>0.05$), but significantly lower than when seed was grown at Meath Park in 2012 (172%) ($p<0.05$).

Similar to FC, foam stability (FS) was found to be similar in magnitude across the genotypes (average = 65%) ($p>0.05$); however, some environmental influence was observed due to location and year of harvest (Figure 4.9B). Values for FB9-4 were similar irrespective of year (2011, 2012) or location (Arborg, Edmonton, Outlook and Rosthern), and so too for Snowbird (Lacombe, Melita, Outlook and Saskatoon-Sutherland) and CDC Snowdrop (Edmonton, Meath Park, Rosthern and Saskatoon-Sutherland) ($p>0.05$). However, FS of the isolates prepared from CDC Fatima grown at Outlook in 2012 (FS = 58%) was similar to that of isolates from seed grown at Lacombe in 2011 or Arborg in 2011 (63%) ($p>0.05$), but significantly lower than when seed was grown at Saskatoon-Preston in 2012 (67%) ($p<0.05$). Similar FS values were observed for isolates prepared from SSNS-1 when seed was grown at Outlook in 2012 or Arborg in 2011 (FS = 61%) ($p>0.05$), and values were observed to be significantly lower than when seed was

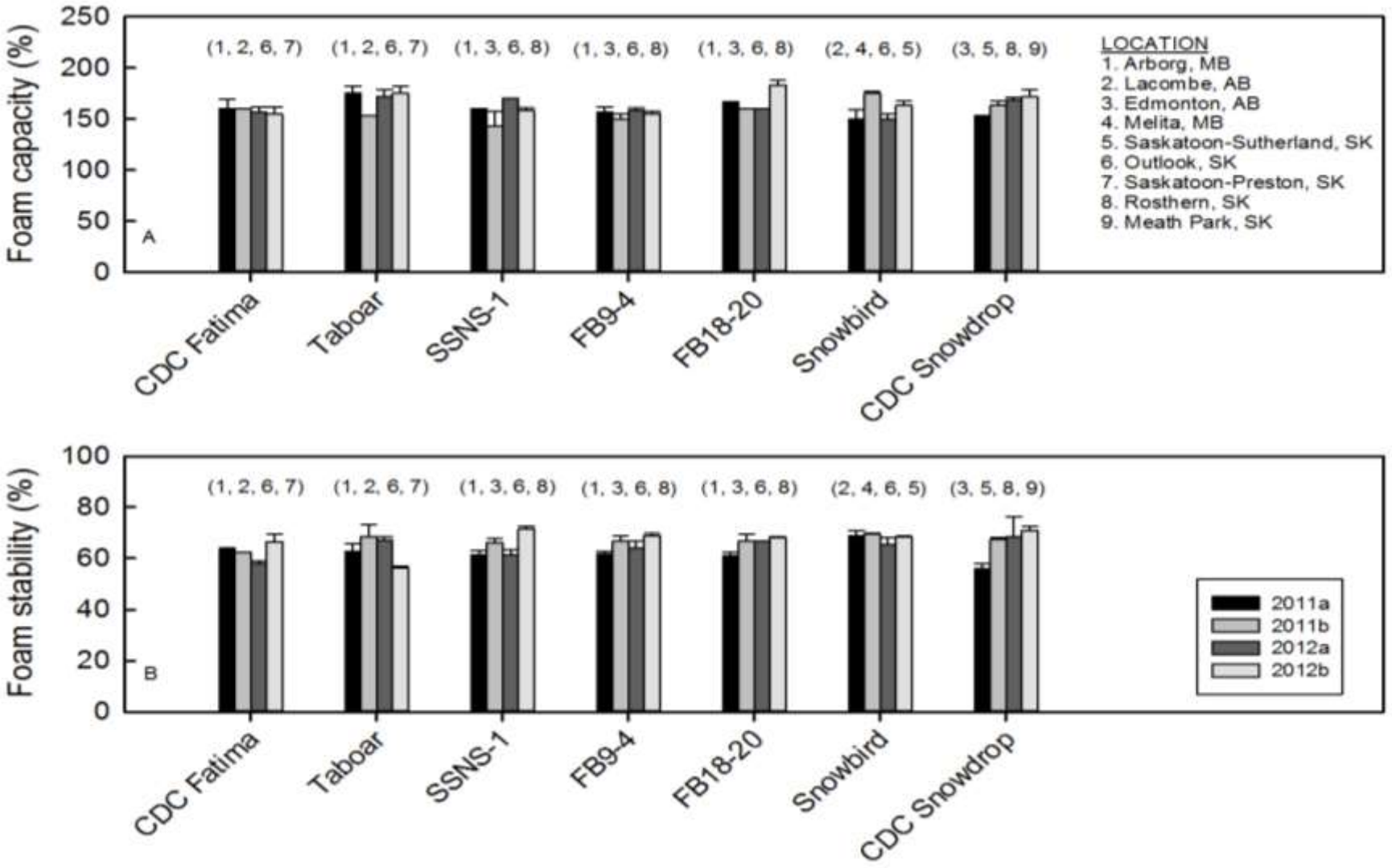


Figure 4.9. Foaming capacity (A) and stability (B) of faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n=2).

grown at Rosthern in 2012 (72%) ($p<0.01$). Foam stability (FS) of isolates prepared from seed of FB18-20 when grown at Rosthern in 2012 (FS = 68%) was similar to that of isolates from seed grown at Outlook in 2012 (67%) or Edmonton in 2011 (67%) ($p>0.05$), but significantly higher than when grown at Arborg in 2011 (61%) (Figure 4.9B) ($p<0.05$). Foam stability values for isolates prepared from Taboar when seed was grown at Saskatoon-Preston in 2012 (FS = 56%) were similar to isolates prepared from seed grown at Arborg in 2011 (63%) or Edmonton in 2012 (67%) ($p>0.05$), and significantly lower than when seed was grown at Lacombe in 2011 (69%) ($p<0.05$).

4.6.5 Protein solubility

Protein solubility is the amount of protein solubilized in the supernatant obtained after centrifugation of a protein solution relative to the total amount of protein present in the original material (Boye et al., 2010b). It is an important determinant of the suitability of a protein material to be used as an emulsifier, foaming agent or thickener in food products. The solubility (Sol) of isolates prepared from faba bean was similar in magnitude across genotypes ($p>0.05$), and ranged between ~75% and ~88%, with a mean of 81% (Figure 4.10). However, environmental effects were observed in response to location and year of harvest within all genotypes. For example, all of the four locations where CDC Fatima was grown had significantly different Sol values, Saskatoon-Preston in 2012 (Sol = 99%), Arborg in 2011 (91%), Lacombe in 2011 (85%) and Outlook in 2012 (65%) ($p<0.01$). The Sol of isolates prepared form Taboar grown at Arborg in 2011 (Sol = 79%) or Outlook in 2012 (82%) were similar in magnitude ($p>0.05$), but significantly higher than when grown at Lacombe in 2011 (66%) or Saskatoon-Preston in 2012 (72%) ($p<0.01$). For FB18-20, Sol values were similar when seed was grown at Outlook in 2012 (Sol = 80%) or Edmonton in 2011 (81%) ($p>0.05$), and significantly lower than when grown at Rosthern in 2012 (88%) or Arborg in 2011 (92%) ($p<0.01$). Similar Sol values were obtained for SSNS-1 when grown at Outlook in 2012 (72%) or Edmonton in 2011 (72%) ($p>0.05$), which were significantly lower than when grown at Arborg in 2011 (88%) or Rosthern in 2012 (96%) ($p<0.01$). The Sol of FB9-4 was similar in magnitude when seed was grown at Arborg in 2011 (77%), Outlook in 2012 (79%) or Rosthern in 2012 (80%) ($p>0.05$), and significantly higher than when grown at Edmonton in 2011 (Sol = 69%) ($p<0.01$). The Sol of Snowbird was similar in magnitude when seed was grown at Saskatoon-Sutherland in 2012

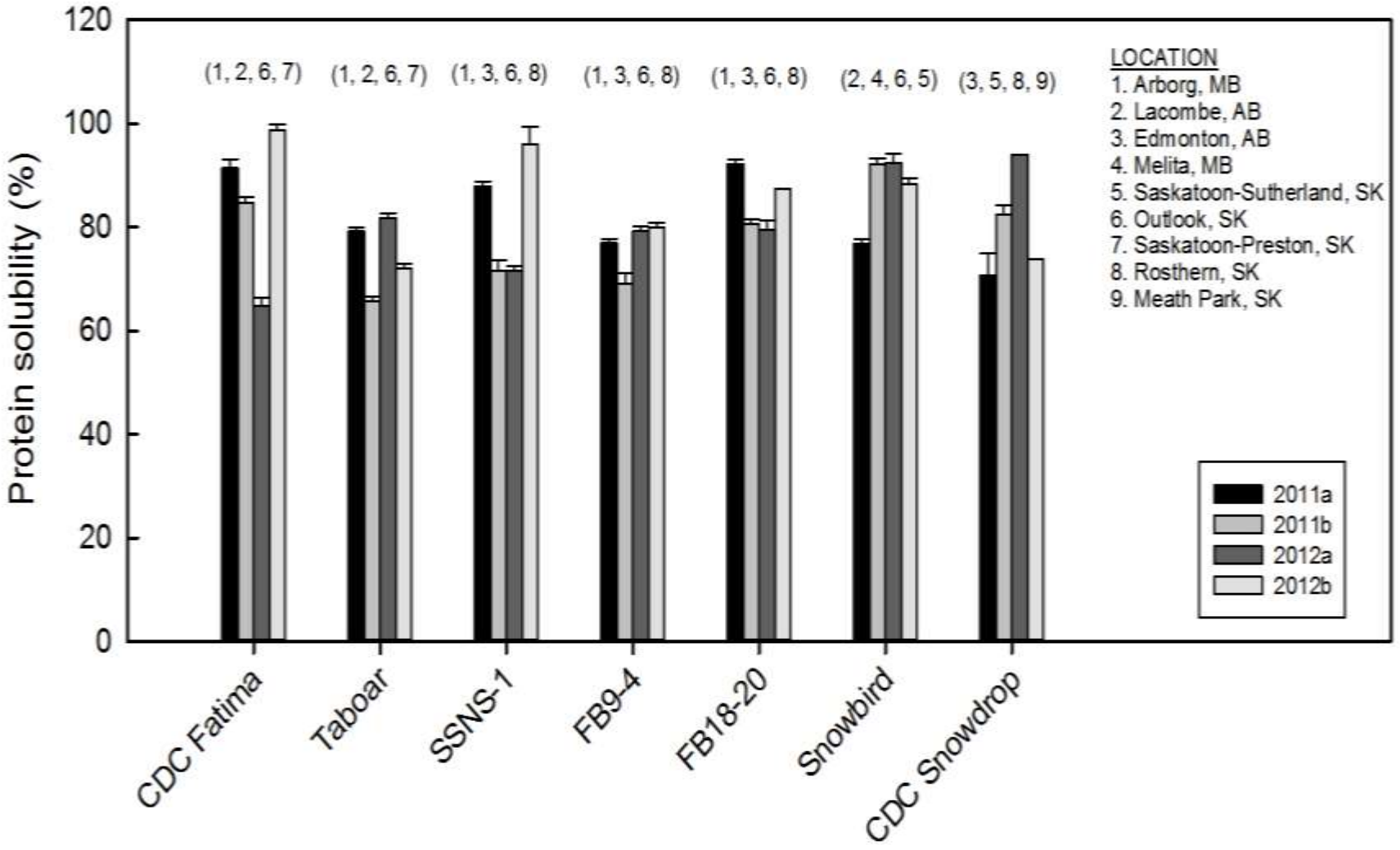


Figure 4.10. Protein solubility (%) of faba bean protein isolates as a function of genotype, location and year at pH 7.0. Data represent the mean ± one standard deviation (n=2).

(88%), Melita in 2011 (92%) or Outlook in 2012 (92%) ($p>0.05$), and significantly higher than when grown at Lacombe in 2011 (Sol = 77%) ($p<0.01$). The Sol of CDC Snowdrop when grown at Rosthern in 2012 (Sol = 94%) was significantly higher than when grown at Edmonton in 2011 (71%), Meath Park in 2012 (74%) or Saskatoon-Sutherland in 2011 (82%) ($p<0.01$).

4.7 G x E relationships

A G x E (Genotype x Environment) assessment was conducted for only three faba bean genotypes, SSNS-1, FB9-4 and FB18-20, since these genotypes were grown at the same locations (Arborg, Edmonton, Outlook and Rosthern) in 2011 and 2012. For the G x E assessment, a G x L (Genotype x Location) analysis, and a G x Y (Genotype x Year) analysis were performed using a two-way ANOVA.

a) Impact of genotype and location

The two-way ANOVA analysis revealed that with the exception of flour crude fat, a significant G x L interaction was present for all of the faba bean flour and isolate proximate components ($p<0.01$). Differences also were significant due to the main effects of genotype and location ($p<0.01$). A significant G x L interaction also were detected for OHC, FC, EC and Sol ($p<0.01$), whereas for all other functional properties (FS, CS, EAI and ESI) it was not present ($p>0.05$). For FS and EAI, significant differences for the main effects only were observed due to the effect of location ($p<0.01$), for CS due to the effect of genotype ($p<0.01$), and for ESI due to the effect of both genotype and location ($p<0.05$). In the case of physicochemical properties, a significant G x L interaction was observed only for SH ($p<0.01$) and not for ZP, ST or IT ($p>0.05$). Moreover, ZP and ST also did exhibit significant differences due to genotype or location ($p>0.05$), whereas IT was influenced by location ($p<0.01$). Interestingly, both L/V and colour characteristics of faba bean flour and isolates were found to have significant G x L interactions ($p<0.01$), where colour was also influenced by the main effects of location and genotype while L/V had only a significant effect of location ($p<0.01$).

b) Impact of genotype and year

A two-way ANOVA analysis showed a significant G x Y interaction only for flour ash ($p<0.01$) whereas for all other faba bean flour and isolate proximate components it was not

present ($p>0.05$). The main effects of both genotype and year were found to be significant for all flour proximate components ($p<0.01$) but not for isolate proximate components ($p>0.05$). Except for EC, a significant G x Y interaction was not observed for any of the functional properties (CS, EAI, ESI, FC, FS, Sol) ($p>0.05$). Overall, the effect of genotype was more prominent for functional properties than the effect of year. For instance, the differences in functional properties due to year were significant for only FC and ESI, whereas the effect of genotype was significant for FC, EC, CS and ESI. The other properties (OHC, FS, EAI and Sol) did not exhibit any significant differences due to either year or genotype ($p>0.05$). For the physicochemical properties, a G x Y interaction along with the main effects of genotype and year were found to be non-significant ($p>0.05$). For colour characteristics, with the exception of faba bean flour and isolate a* values, a G x Y interaction was not found ($p>0.05$). A significant G x Y interaction was present for the L/V of faba bean flour but not for faba bean isolates ($p<0.05$), and the main effects of genotype and year on L/V properties also were non-significant ($p>0.05$).

4.8 Structure-function relationships

A Pearson correlation analysis was performed to observe whether any relationships existed between the legumin: viclin (L/V) ratio (of the isolates), colour characteristics, seed size or physicochemical characteristics and each functional property (Table 4.4). Except for colour characteristics, there were very few significant correlations. For example, 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 higher the legumin content, the less highly charged the proteins, and the L/V ratio has a larger effect on surface charge than on functionality. The latter would be complicated by the conformation of the protein and its interactions with the solvent. Foam stability ($r = 0.54$, $p<0.05$), creaming stability ($r = 0.46$, $p<0.05$) and solubility ($r = 0.44$, $p<0.05$) were found to be positively correlated with zeta potential. Seed size was not found to be significantly correlated with any of the colour or proximate component of faba bean protein isolates. Some of the functional properties were found to be significantly correlated with isolate colour properties. For example, foam stability was found to be negatively correlated with isolate L* value ($r = -0.40$, $p<0.05$), isolate a* value ($r = -0.55$, $p<0.05$) and isolate b* value ($r = -0.54$, $p<0.05$). EAI was not significantly correlated with any functional or physicochemical properties whereas ESI was only negatively correlated with

Table 4.4 Pearson correlation coefficients (r) for physicochemical, colour, seed size, functional, proximate, and L/V properties of faba bean flour and protein isolates from all genotypes.

	Seed size	Isolate L*	Isolate b*	Isolate a*	IY	Isolate ash	L/V isolate	OHC	FC	FS	EC	CS	ESI	EAI	ZP	IF	ST	IT
Isolate L*	-0.02																	
Isolate b*	-0.24	0.73*																
Isolate a*	0.04	0.44*	0.60*															
IY	-0.18	-0.01	0.01	-0.27														
Isolate protein	-0.14	0.21	0.16	0.20	0.02													
Isolate ash	-0.05	-0.21	-0.03	-0.33	0.09													
L/V isolate	0.30	0.43*	0.07	0.17	-0.15	-0.25												
OHC	0.00	0.16	0.15	0.21	-0.30	-0.34	0.11											
FC	-0.13	0.08	0.07	-0.16	0.00	0.40*	-0.27	-0.26										
FS	0.07	-0.40*	-0.54*	-0.55*	0.40*	0.22	-0.06	-0.55*	0.00									
EC	0.02	-0.11	-0.07	-0.11	-0.35	0.52*	0.04	-0.11	0.20	-0.06								
CS	-0.39*	-0.46*	-0.18	-0.29	0.19	0.07	-0.33	-0.39*	0.04	0.24	0.00							
ESI	0.33	-0.47	-0.45*	-0.31	0.26	0.03	0.01	-0.32	0.02	0.37	0.13	0.23						
EAI	-0.29	-0.22	0.13	-0.02	0.28	0.31	-0.02	-0.06	0.07	0.14	-0.13	0.10	0.05					
ZP	-0.11	-0.53*	-0.36	-0.50*	0.30	0.29	-0.39*	-0.37	-0.11	0.54*	0.20	0.46*	0.15	0.02				
IF	0.30	0.11	-0.06	0.03	-0.05	-0.28	0.40*	-0.28	-0.03	0.01	0.02	-0.13	0.16	-0.15	0.06			
ST	-0.13	0.28	0.10	0.28	0.18	-0.35	0.17	-0.25	-0.22	-0.11	-0.28	0.12	-0.04	-0.31	-0.17	0.14		
IT	-0.22	0.01	-0.07	0.06	-0.08	0.15	0.19	0.07	-0.10	-0.30	0.08	-0.02	-0.18	0.08	-0.20	-0.15	0.22	
Sol	0.11	-0.23	-0.26	-0.36	-0.21	0.09	-0.07	-0.38*	0.21	0.34	0.18	0.31	0.17	-0.22	0.44*	0.29	-0.32	-0.38*

* indicates significant correlations at $P<0.05$.

IY, isolate yield; OHC, oil holding capacity; FC, foaming capacity; FS, foam stability; EC, emulsion capacity; CS, creaming stability; ESI, emulsion stability index; EAI, emulsion activity index; ZP, zeta potential; IF, Intrinsic fluorescence; ST, surface tension; IT, interfacial tension; Sol, solubility.

isolate L* value ($r = -0.47$, $p<0.05$) and b* value ($r = -0.45$, $p<0.05$). Correlations for SSNS-1, FB9-4 and FB18-20 were quite different than those for all the genotypes with a number of significant correlations observed in the latter (Table 4.5). Surface tension was observed to be positively correlated with CS ($r = 0.61$; $p<0.05$) and ZP ($r = 0.90$; $p<0.05$), and negatively correlated with OHC ($r = -0.64$; $p<0.05$), whereas IT was negatively correlated with only solubility ($r = -0.61$; $p<0.05$). The L/V of isolates was not found to be significantly correlated with any of the functional or physicochemical properties.

4.9 Principal component analysis (PCA): Scatter plot

PCA models transform a given set of variables into a few variable coordinate systems, while capturing most of the variability from the original data. These sets of new variables are known as principal components. To evaluate the comparative effects among genotypes, years and locations, scatter plots in principal component analysis (PCA) models were studied (Figure 4.11). Variables close to each other affect the PCA model in a similar way and are positively correlated, whereas variables diagonally opposite are assumed to be negatively correlated. Moreover, the farther the variable from one origin, the more influential it is. Snowbird and Snowdrop (zero tannin genotypes) were found to be closely grouped and observed to be more influential as compared to other genotypes. In addition, these zero tannin genotypes were observed to have an opposite effect compared to the other genotypes placed diagonally opposite in the scatter plot. No effect of seed size was seen, since in most of the cases, genotypes having similar seed size are not grouped together. Genotypes grown at the same locations in 2011 and 2012 were found to be grouped together, e.g. CDC Fatima and Taboar; and SSNS-1, FB9-4 and FB18-20. However, Snowbird and Snowdrop although not having similar locations, are grouped suggesting seed composition may play a larger role than environmental effects on faba bean functionality and other properties. The effects of the Edmonton and Lacombe locations from 2011 were found to be similar, but negatively correlated to the similar effects of Rosthern and Outlook in 2012, showing the influence of location and year effects. Furthermore, five different faba bean genotypes grown at Arborg (MB) in 2011 were found to be closely distributed on the scatter plot. Overall, the effect of year was observed to be quite distinct, where 2011 was oppositely placed to 2012 (Figure 4.11).

Table 4.5 Pearson correlation coefficients (r) for physicochemical, colour, seed size, functional, proximate, and L/V properties of faba bean flour and protein isolates from SSNS-1, FB9-4 and FB18-20 genotypes.

	Seed size	Isolate L*	Isolate a*	Isolate b*	IY	Isolate protein	Isolate ash	L/V isolate	OHC	FC	FS	EC	CS	EAI	ESI	ZP	ST	IF	IT
Isolate L*	0.03																		
Isolate a*	-0.15	-0.02																	
Isolate b*	-0.43	0.71*	0.40																
IY	-0.42	0.09	0.11	0.39															
Isolate protein	-0.45	0.12	-0.09	0.35	0.11														
Isolate ash	0.30	0.19	0.07	0.19	0.16	-0.07													
L/V isolate	0.09	0.35	-0.05	-0.09	0.07	-0.18	0.04												
OHC	-0.11	0.17	-0.04	0.08	-0.65*	0.52	0.04	0.00											
FC	0.16	0.23	-0.40	0.17	-0.04	0.18	0.16	-0.14	-0.02										
FS	0.05	-0.19	-0.12	-0.32	0.51	-0.51	0.06	0.29	-0.76*	-0.12									
EC	0.28	-0.05	-0.09	-0.16	-0.02	-0.18	0.59*	0.00	-0.07	0.25	0.14								
CS	-0.74*	-0.08	0.21	0.42	0.49	0.15	-0.41	-0.28	-0.38	0.06	0.13	-0.28							
EAI	-0.32	-0.07	0.19	0.06	0.42	0.51	0.19	0.41	0.08	-0.11	0.22	-0.06	0.03						
ESI	0.42	-0.35	-0.18	-0.45	0.13	-0.42	0.19	0.03	-0.56	0.49	0.61*	0.46	-0.12	0.04					
ZP	-0.14	-0.56	0.10	-0.28	0.28	-0.41	-0.31	-0.32	-0.55	-0.50	0.25	-0.08	0.37	-0.37	-0.02				
ST	-0.30	-0.39	0.23	0.02	0.47	-0.30	-0.35	-0.32	-0.64*	-0.41	0.25	-0.35	0.61*	-0.24	-0.09	0.90*			
IF	0.19	-0.18	-0.11	-0.31	0.05	-0.04	-0.48	0.31	-0.29	0.12	-0.03	-0.05	0.02	-0.03	0.23	0.26	0.21		
IT	-0.03	-0.03	0.70*	0.14	-0.13	0.06	0.27	0.29	0.34	-0.56	-0.28	-0.12	-0.16	0.40	-0.43	-0.08	-0.03	-0.15	
Sol	-0.07	-0.10	-0.57	-0.27	-0.21	-0.38	-0.31	-0.01	-0.14	0.44	0.19	-0.11	0.20	-0.46	0.31	0.09	0.07	0.08	-0.61*

* indicates significant correlations at P<0.05.

IY, isolate yield; OHC, oil holding capacity; FC, Foaming capacity; FS, foam stability; EC, emulsion capacity; CS, creaming stability; EAI, emulsion activity index; ESI, emulsion stability index; ZP, zeta potential; IF, Intrinsic fluorescence; ST, surface tension; IT, interfacial tension; Sol, solubility.

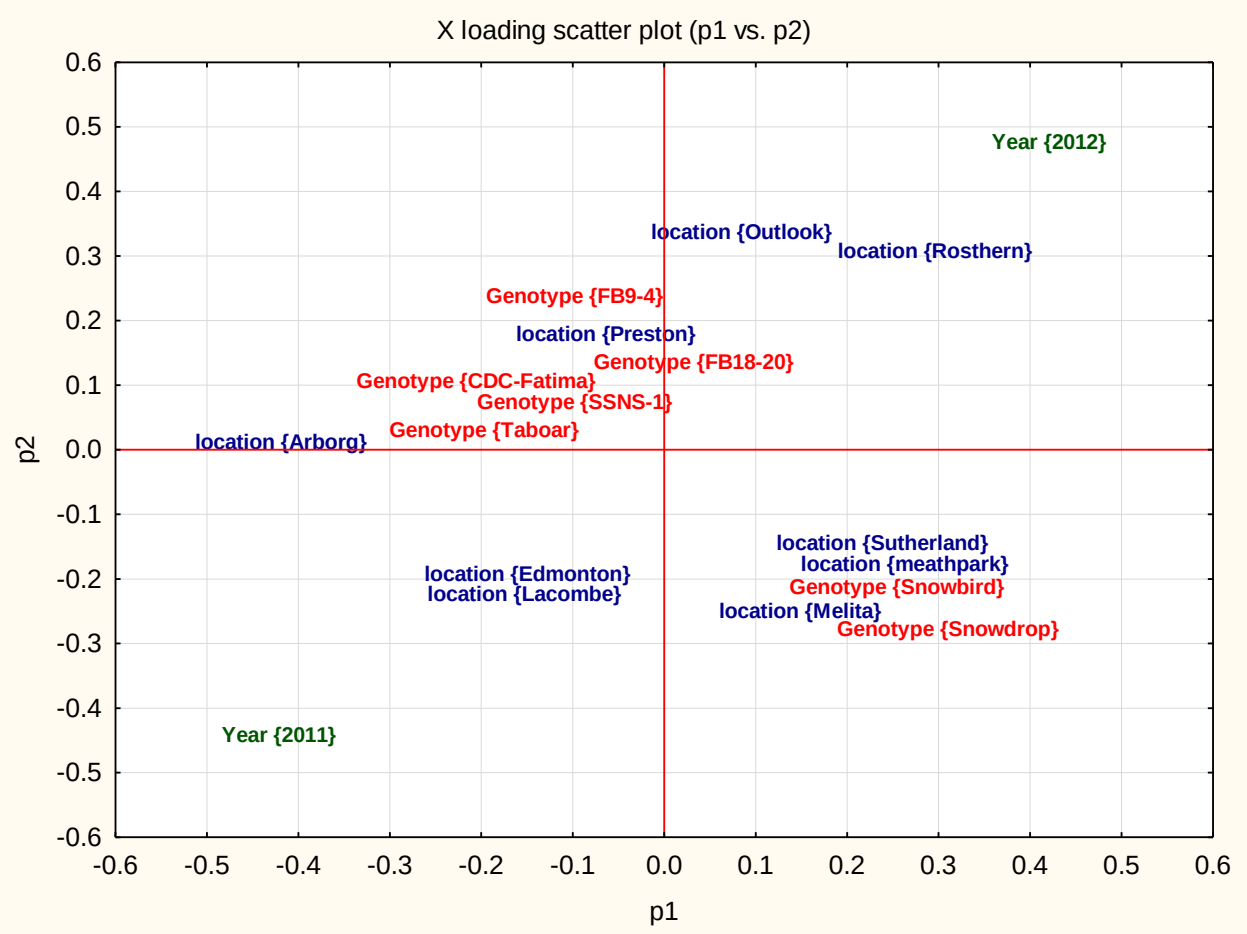


Figure 4.11. The Scatter plot of the first two principal components (p1 and p2), depicting the distribution of all faba bean genotypes, locations and years in the PCA model.

4.10 Comparison to commerical isolates

The functional properties of the faba bean isolates were compared with those of commercial isolates, including those from soybean, pea, egg, whey and wheat. It should be noted that commercial production of isolates may result in varying protein functionality depending on the processing technique or conditions used. For example, the use of spray drying in commercial production of protein isolates in comparison to freeze drying was reported to result in a lower protein solubility (Otegui et al., 1997). The relatively large standard deviations in some cases reflect variability due to environment (year and/or location) as discussed previously. The mean foaming capacity (FC) of faba bean protein isolates (162%) was found to be significantly higher than that of the egg protein isolate (112%) ($p<0.01$), and similar to those of isolates from pea

(150%) and soybean (157%) ($p>0.05$), and lower than those of isolates from wheat (223%) and whey (228%) ($p<0.01$) (Table 4.6). On the contrary, the mean foam stability (FS) of faba bean isolates (65%) was found to be similar to the FS of all of the commercial isolates (pea = 56%, soy = 58%, whey = 69%, egg = 73% and wheat = 76%) ($p>0.05$). The mean oil holding capacity (OHC) of faba bean isolates (5.7 g/g) was found to be higher than those of all of 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 protein solubility of the faba bean isolate (81%) was found to be higher than those of isolates from wheat (11%), pea (20%) and soybean (31%) ($p<0.05$), but lower than those of isolates from egg (88%) and whey (89%) ($p<0.05$). The mean emulsion capacity (EC) of faba bean isolates (184 g/g) was found to be similar in magnitude to those of isolates from soybean (175 g/g), pea (181 g/g) and egg (184 g/g) ($p>0.05$), lower than that of the isolate from whey (206 g/g) ($p<0.01$), and higher than that of the isolate from wheat (94 g/g) ($p<0.01$). In contrast, the mean creaming stability (CS) of faba bean isolates (94%) was found to be similar in magnitude to those of isolates from whey (89%) and egg (96%) ($p>0.05$), lower than that of the isolate from soybean (100%), and higher than those of isolates from pea (50%) and wheat (62%) ($p<0.01$).

The mean emulsion activity index (EAI) of faba bean isolates (13.0 m^2/g) was found to be similar to those of isolates from egg (11.5 m^2/g), whey (14.5 m^2/g) and soy (14.6 m^2/g) ($p>0.05$), and higher than those of isolates from wheat (1.2 m2/g) and pea (1.7 m2/g) ($p<0.01$). Similarly, the mean emulsion stability index (ESI) of faba bean isolates (10.7 min) was found to be similar to those of isolates from egg (11.6 min), pea (11.9 min) and soybean (12.0 min) ($p>0.05$), but lower than those of isolates from whey (13.0 min) and wheat (13.1 min) ($p<0.05$). These results indicate that protein isolates having better functionality may be prepared from faba bean and might be used effectively in a variety of food applications. For instance, a higher protein solubility profile for a faba bean isolate might be advantageous in protein drinks and milk replacers, whereas a higher OHC may play an important role in meat products by reducing fat loss and improving binding properties. Moreover, the better emulsifying properties of faba bean isolates indicate their potential significance in the production of bread, meat sausages, salad dressings and dairy products, whereas isolates with better foaming properties might be employed in the production of whipped toppings, and confectionery products (Hayat et al., 2014).

Table 4.6. Foaming capacity (FC) and stability (FS), emulsion capacity (EC), creaming stability (CS), oil holding capacity (OHC), emulsion activity (EAI) and stability (ESI) indices, and protein solubility (Sol) for faba bean protein isolates as a function of genotype. Data for the faba bean protein isolates represents the mean value for each genotype (2 locations x 2 years x duplicate measurements) ± one standard deviation (n = 8). Data for the commercial protein isolates represents the mean value of duplicate measurements ± one standard deviation (n = 2).

Genotype	FC (%)	FS (%)	EC (g/g)	CS (%)	OHC (g/g)	EAI (m^2/g)	ESI (min)	Sol (%)
a) Faba bean protein isolates								
CDC Fatima	158 ± 3	63 ± 4	189 ± 3	95 ± 1	5.7 ± 0.7	12.1 ± 0.6	10.7 ± 0.6	84.9 ± 14.6
Taboar	169 ± 10	64 ± 6	184 ± 4	93 ± 2	5.9 ± 1.0	12.5 ± 1.3	10.6 ± 0.1	74.8 ± 7.3
SSNS-1	158 ± 11	65 ± 5	177 ± 3	95 ± 1	5.9 ± 1.0	13.6 ± 0.6	10.6 ± 0.1	81.8 ± 12.2
FB9-4	155 ± 4	65 ± 3	178 ± 8	92 ± 2	5.6 ± 0.5	12.7 ± 1.5	10.7 ± 0.2	76.3 ± 4.9
FB18-20	168 ± 11	65 ± 3	186 ± 14	92 ± 2	5.8 ± 0.9	12.8 ± 1.3	11.0 ± 0.3	85.0 ± 6.0
Snowbird	160 ± 12	68 ± 2	186 ± 14	96 ± 1	5.7 ± 0.3	13.0 ± 0.5	11.0 ± 0.3	87.5 ± 7.3
CDC Snowdrop	164 ± 8	66 ± 7	186 ± 11	95 ± 1	5.4 ± 0.7	14.3 ± 0.8	10.7 ± 0.1	80.3 ± 10.4
b) Faba bean and commercial protein isolates								
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}	58 ± 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}	56 ± 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}

No significant differences across genotypes were observed in part (a) ($p>0.05$), and means in each column followed by different letters are significantly different ($p<0.05$) as shown in part (b).

5. DISCUSSION

Faba bean is one of the oldest crops used as a source of protein in the human diet, and for feed purposes for animals. It also represents a valuable source of dietary fibre, essential vitamins and minerals. Faba bean is consumed abundantly in Africa, Middle Eastern countries, and countries such as India and China in the form of boiled beans or curries prepared with other locally grown crops. However, allergen concerns may arise upon the consumption of faba bean in certain populations (Mur-Gimeno et al., 2007; Damiani et al., 2011). Kumar et al. (2014) reported faba bean hypersensitivity in mice led to increased levels of histamine and IgE (immunoglobulin E) and severe signs of anaphylaxis. Despite these allergic studies, there are still very few cases of fava bean allergy reported in humans. Faba bean also contains glycosides (vicine and convicine) which are considered to be the root cause of favism, a type of haemolytic anemia (McMillan et al., 2001). Individuals who are deficient in G6PD enzyme (glucose-6-phosphate dehydrogenase) are particularly affected by the consumption of uncooked faba bean since NADPH (reduced nicotinamide adenine dinucleotide phosphate) is not produced in sufficient quantities to combat the oxidative stress produced by these glycosides (McMillan et al., 2001). Faba bean genotypes which are low in vicine and convicine may prove to be advantageous in providing high nutritional performance in humans (Vilarino et al., 2009; Duc et al., 2011). In this study, all of the faba bean genotypes examined were considered to have normal levels of vicine. However, Vioque et al. (2012) reported that the content of glycosides (measured using RP-HPLC), including vicine and convicine, is reduced significantly (to a level of <1% of the original flour) during the production of protein isolates using an isoelectric precipitation method. Apart from protein extraction, there are other methods reported in the literature which reduce the content of anti-nutritional factors, including soaking, dehulling, extrusion, heat treatment, enzymatic or genetic modification, and fermentation (van der Poel, 1990; Alonso et al., 2000; Granito et al., 2002; Luo et al., 2009; Crepon et al., 2010; Jezierny et al., 2010).

Legumes such as faba bean are important sources of natural antioxidants with health promoting benefits. These antioxidant properties are mainly because of high total phenolic content from phenols, flavonoids, tannins, lignins, etc. (Shahidi and Naczk, 2004). Faba bean

contains more than twice the total polyphenol content present in commercial pulses such as pigeon pea and field pea, and also was reported to have nine and fifteen times, respectively, more antioxidant activity than these legumes (Vioque et al., 2012). However, these important properties may be removed during the defatting and wet extraction process, because of the high solubility of polyphenols in extraction solvents such as water, acetone, ethanol and methanol (Sun and Ho, 2005). This shows one of the disadvantages of using a wet extraction method to produce protein isolates or concentrates as compared to dry extraction methods, which does not require any solvents. Moreover, wet extraction methods are more complex, and cost more money because of higher energy and water input than dry extraction methods (Schutyser and van der Goot, 2011). Although wet extraction methods give a higher protein content, they may also result in a loss of protein functionality due to processing conditions such as change in pH and the drying method (Pelgrom et al., 2013). It has already been established that the genetic diversity of faba bean is closely related to its geographical origin, growth habit and ecological conditions (Robertson and El-sherbeeny, 1991; Polignano et al., 1993; Zong et al., 2009; Duc et al., 2010; Zong et al., 2010). For example, Wang et al. (2012) reported that the faba bean varieties grown in North China and Europe had the highest genetic variation, and those grown in Central China and Africa had the lowest genetic variations. It also was observed from a PCA scatter plot that faba bean germplasm grown in China in the winter was grouped separately from that grown in China in the spring (Wang et al., 2012). Similarly, the PCA scatter plot in the current study showed that the effect of location where seed was grown was more profound than the genotypic or year effect. For example, five different faba bean genotypes all grown at Arborg (MB) in 2011 were found to be closely located on the scatter plot. These results are in accordance with one-way ANOVA results where faba bean genotype was not found to have a significant influence on most of the parameters examined in this study, but considerable effects due to environment were observed. The effect of year from the PCA model was observed to be most distinct, in that 2011 was placed far from and opposite to 2012. However, using a G x E assessment, the overall effect of location was observed to be much higher than the effect of year. Thus, due to limited seed availability, it is difficult to say whether the effect of year from PCA model is a true year effect or because of the location in which seed was grown for those years.

5.1 Colour characteristics and proximate composition of faba bean flours and isolates

The colour of protein isolates is an important attribute which needs to be controlled and optimized for final product evaluation and consumer acceptance. Various methods has been studied which reduce colour content including dehulling of seed (Onigbinde and Onobun, 1993), protein extraction (Sosulski et al., 1988), extraction pH (Adebowale et al., 2007), defatting of flour (Toews and Wang, 2013), and final particle size (Han and Khan, 1990). Faba bean flour colour differed significantly for L* and b* values, but flour were similar with respect to the a* parameter. In contrast to the flours, all protein isolates were similar in colour, as evidenced by their similar L*, a* and b* values, suggesting the extraction process and defatting procedure removed most of the phenolic compounds and other pigments that remained after dehulling. This is also supported from the study of Toews and Wang (2013) in which defatted chickpea concentrates were observed to be brighter and having less red and yellow colour as compared to non-defatted ones. Colour characteristics of isolates were not found to be significant correlated with isolate proximate composition but found to be negatively correlated with the functional and physicochemical properties. For example, ZP and CS were negatively correlated with isolate L* value ($r = -0.53$, $p<0.05$) and isolate L* value ($r = -0.46$, $p<0.05$), respectively. And, foam stability was negatively correlated with isolate L* value ($r = -0.40$, $p<0.05$), isolate a* value ($r = -0.55$, $p<0.05$) and isolate b* value ($r = -0.54$, $p<0.05$). It is unclear how colour properties affect protein functionality, but a possible explanation could be the interaction of chemical structure of colour pigments with protein molecules which in turn could have influential effect on protein functionality.

The composition of the faba bean flours indicated that protein, ash and crude fat levels were similar irrespective of the genotype. Protein, ash and crude fat levels (on a moisture free basis) in the flour were found to be ~32%, ~3.4% and ~1.3%, respectively. Similar to the flour, the levels of protein and ash in the isolates were similar across genotypes at levels of ~94.0% and ~5.8%, respectively. Protein levels were found to be higher than those of the commercial isolates used for comparative purposes, i.e., on a moisture free basis, pea (80.4%), soy (83.4%), wheat (91.1), egg (81.7), and whey (92.8). The higher ash contents of the isolates relative to the flours reflect the use of the pH adjustments during the extraction/precipitation process (from pH 7.0 to 9.5/from pH 9.5 to 4.5). No desalting step was employed during this process. The negligible levels of crude fat in the isolates were caused by the defatting of the flour prior to isolate

preparation. Defatting increases the protein contents of isolates and aids in the extraction process by reducing protein-lipid interactions which could have the effect of lowering protein solubility (Levya-Lopez et al., 1995; Can Karaca et al., 2011). Defatting also results in improvement in the nutritional and functional properties of protein. For example, Toews and Wang (2013) showed that for three chickpea genotypes, concentrates made from defatted flours had higher trypsin inhibitor activity (TIA), higher levels of protein, ash and starch, and improved WHC, FC and FS as compared to those made from non-defatted flours, irrespective of genotype.

5.2 Extraction efficiencies of isolate production

The protein contents and protein yields of the protein isolates were comparable to values in the literature for faba bean protein isolates. Qayyum et al. (2012), Fan and Sosulski (1974) and McCurdy and Knipfel (1990) produced faba bean protein isolates using alkaline extraction-isoelectric precipitation, and reported protein contents of ~77.6%, ~93.1% and ~92.7%, respectively, and protein yields of ~67.6%, ~80.2% and ~62.6%, respectively. The isolate yields also were found to be comparable to values in the literature for isolates produced by alkaline extraction-isoelectric precipitation and reported to be ~19.7% (Qayyum et al., 2012) and ~28.2% (Fan and Sosulski, 1974), respectively. The slightly higher values in the present study may be attributed to the second alkaline extraction of the feedstock.

5.3 Protein composition of flours and isolates

The gel electrophoresis profiles of both the faba bean flour and isolate samples were similar and the four major bands of α-legumin, β-legumin, vicilin and convicilin were evident. This protein profile was similar to that of pea as reported in the study by Nikolic et al. (2012). Other techniques used to separate the protein fractions for identification purposes include chromatographic techniques such as high and low pressure liquid chromatography. These techniques have the added benefit of being able to better purify the sample of lower molecular weight albumins (Mertens et al., 2011; Klassen and Nickerson, 2012; Vioque et al., 2012). Information in the literature on convicilin is limited, however it has been shown to be immunologically similar to vicilin based on the study by Croy et al. (1980). Legumin:vicilin (L/V) ratios for faba bean were found to be independent of genotype for both the flours and the isolates, and shifted from 3.8 to 4.5 during the alkaline extraction-isoelectric precipitation

process, indicating that the pH and ionic conditions selected preferentially for higher amounts of legumin in the isolates. These findings suggest that processing will influence the protein profile of the isolate, irrespective of the composition of the feedstock. It is suggested that an optimized wet extraction process could be identified by adjusting the pH, salt type and ionic strength to select for legumin or vicilin. This in turn would alter the functionality of the isolate.

Pradhan et al. (2014) found high genetic variation in the amount of albumin and globulin proteins from different faba bean genotypes grown in different climatic zones of Bihar, India. These genetic variations were probably due to polymorphism in protein bands which are specific to each genotype. These polymorphic bands may be one source of variation in the L/V ratio between genotypes of legume species, as observed in this study, although probably not significant in nature. The L/V ratios for the isolates were slightly higher than values obtained for another faba bean globulin fraction prepared using a salt extraction process (2.1-3.6), suggesting that many extrinsic (environmental) and intrinsic (pH, salt and others) factors may influence the ratio (Gatehouse et al., 1980). Interestingly, in the present study the L/V ratio varied significantly in response to the environment (location and year) within each genotype, with the exception of the zero-tannin genotype Snowbird for faba bean flours, and CDC Snowdrop for faba bean protein isolates. This suggests that at least in terms of protein composition, zero-tannin varieties may represent a more constant feedstock. It also was discovered from the PCA scatter plot that zero tannin genotypes (Snowbird and Snowdrop) were more influential and were affecting the PCA model in the opposite way as compared tannin-containing genotypes. Moreover, these genotypes were grouped together despite having different seed sizes, suggesting compositional properties may be of more importance than phenotypic characters in relating protein functionality and other parameters. It is presumed that the presence of tannins and/or distribution of tannins in the extraction solution could impact the solubility of the legumin or vicilin proteins, and hence may result in more variability within the data from different environments. However, no significant differences in solubility were observed between zero-tannin (Snowbird and CDC Snowdrop) and tannin-containing genotypes (CDC Fatima, FB9-4, FB18-20 and SSNS-1). Low tannin content in faba bean also is reported to be associated with higher protein content and had a positive effect on nutritional value and protein digestibility (Crepon et al., 2010; Micek et al., 2015). However, it was found from this study that low tannin varieties had slightly lower protein contents in both flours and isolates, and slightly higher ash contents in flour and isolates

compared to tannin-containing genotypes, which indicates that agronomic or environmental factors also may be important in determining the nutritional quality of faba beans. Tannin levels in response to the environment for each genotype were not monitored in this study.

In contrast to the study of Mertens et al. (2011) and Martensson (1980), the L/V ratio of both faba bean flours and isolates were found to not be correlated with protein content. Moreover, the L/V ratio of faba bean isolates was found to not be correlated with any of the functional attributes, and was found only to be negatively correlated with zeta potential (ZP) ($r = -0.39$; $p<0.05$) and positively correlated with surface hydrophobicity (SH) ($r = 0.40$; $p<0.05$). This correlation indicates that a higher legumin content would lead to an isolate that is more hydrophobic and less charged. A number of studies have reported that vicilin-rich isolates had better protein extractability, solubility, and gelling and emulsifying properties than legumin-rich isolates (Bora et al., 1994; Cserhalmi et al., 1998; Kimura et al., 2008; Barac et al., 2010). In general, proteins with higher charge and less hydrophobicity tend to be more soluble (Jung et al., 2005; McClements, 2005; Can Karaca et al., 2011). However, the extent to which hydrophobic moieties are exposed can have an impact on the interaction of proteins with the oil-water or air-water interface in emulsions and foams, respectively, and in the level of aggregation and gelation. Depending on the functional attribute, protein isolates that have lower or higher L/V ratios may be of interest. Understanding the balance between charge and hydrophobicity in proteins plays a major role in predicting functionality. In the present study, differences in L/V ratios between genotypes could not be correlated to the functional attributes, suggesting that either the variability in data arising from environmental effects prevented clear delineation within the data set, or other factors such as the conformation, mobility or rigidity of proteins in solution are important within the structure-function relationship.

5.4 Functional properties of isolates and comparison with commercial isolates

Protein functionality signifies how individual protein molecules behave in solution. Protein ingredients, when added to food systems, can provide desirable functional attribute such as solubility, textural properties, water and oil binding, emulsification and foaming. Functional properties of faba bean isolates obtained in this study were compared to those of commercial isolates prepared from whey, soy, pea, egg or wheat. However, it should be noted that there may be wide variation in functionality of these individual commercial isolates due to the extraction

method (isoelectric precipitation, ultrafiltration, salt extraction) or processing conditions used in their preparation (spray or freeze-dried, pH, temperature, ionic conditions). For instance, in the study by Vose (1980), faba bean and pea protein isolates prepared by ultrafiltration were found to be 22% and 15% more soluble than those prepared by isoelectric precipitation.

5.4.1 Oil holding capacity

Oil holding capacity (OHC) is a measure of the amount of oil bound per gram of protein (g/g). The OHC is of great importance from an industrial point of view since it may provide a useful index of the ability of protein to prevent fluid leakage from a product during processing, and it reflects useful attributes such as emulsion capacity (EC), a desirable property in products such as mayonnaise (Escamilla-Silva et al., 2003; Kiosseoglou and Paraskevopoulou, 2011). For a protein to have a higher OHC, a large number of non-polar amino acids available to bind oil molecules are required. However, no significant correlation was observed between OHC and surface hydrophobicity (SH) of faba bean protein isolates. The faba bean protein isolates were found to have significantly higher OHCs (5.7 g/g) in comparison to all of the commercial isolates tested (1.1-2.3 g/g) ($p<0.01$). Furthermore, the OHCs of faba bean isolates obtained in this study were higher than those reported in the literature for faba bean isolates produced using an isoelectric precipitation method, with values of 1.8 g/g (Fernandez-Quintela et al., 1997) and 2.3 g/g (Vioque et al., 2012).

In contrast, the results showed that the water holding capacity (WHC) of faba bean protein isolates was relatively low, and in most cases the faba bean protein isolates were completely dispersed, even when centrifuged at a higher speed and tested at different protein concentrations. Thus, WHC was not assessed further and results are not shown in this study. Possibly another WHC method could be attempted in the future to better assess the protein ingredient. However, WHC of other faba bean isolates have been reported to have values comparable to those from other legume sources. For example, Fernandez-Quintela et al. (1997) and Vioque et al. (2012) reported WHC of 1.8 g/g and 2.5 g/g, respectively, for faba bean protein isolates, and these values were comparable to the WHC of pea (1.7 g/g) and soy (1.3 g/g) isolates (Fernandez-Quintela et al., 1997). There are a number of factors which influence the WHC of proteins, including the conformational characteristics, hydrophilic-hydrophobic balance of amino acids, carbohydrate content, temperature, thermodynamic properties, pH, ionic strength and

solubility of protein (Chavan et al., 2001). Differences in the WHC of faba bean isolates obtained in this study compared to values reported in the literature could be a function of one or more of these parameters. The low WHC of faba bean isolates obtained in this study suggests that faba bean isolates may not be suitable for making meat products where protein works as a water binder and aids in reducing cooking losses and product shrinkage.

5.4.2 Emulsifying capacity, creaming stability, emulsifying activity and stability indices

Proteins can be used as emulsifiers owing to their amphiphilic nature (containing both hydrophobic and hydrophilic amino acid groups) and their ability to form viscoelastic films around oil droplets (Foegeding and Davis, 2011). Protein molecules migrate to oil-water interfaces in an emulsion and re-orient themselves with their hydrophobic groups toward the oil phase and their hydrophilic groups toward the aqueous phase (Walstra, 2003). As more protein molecules adsorb at the oil-water interface, a viscoelastic layer is formed around the oil droplet which provides creaming stability via electrostatic repulsion and steric stabilization (Tcholakova et al., 2006). In this study, emulsifying properties [emulsion capacity (EC), creaming stability (CS), and emulsion activity and stability indices (EAI and ESI)] were evaluated for faba bean isolates and for commercial protein isolates (whey, wheat, soy, pea and egg). With the exception of EAI and ESI, differences due to genotype and environment were observed in all of the emulsifying properties of faba bean protein isolates.

Emulsion capacity (EC) is the maximum amount of oil that can be emulsified before an oil-in-water emulsion is converted to a water-in-oil emulsion, whereas creaming stability (CS) is a measure of the resistance to emulsion separation due to flocculation and aggregation of oil droplets over time (Tcholakova et al., 2006). Legume proteins having higher emulsion capacities and creaming stabilities would be better candidates for protein emulsification applications. In this study, except for the whey protein isolate, the mean EC of faba bean isolates was comparable to those of commercial isolates, and the CS was ~90% or higher for faba bean, soy, egg and whey isolates; however, for pea protein isolate CS was just 50%.

The EAI and ESI values describe the ability of a protein to form an emulsion (Hill, 1996). The EAI is a measure of the interfacial area stabilized per gram of protein, calculated by analyzing the turbidity of diluted emulsions, whereas ESI is a measure of the stability of the same emulsion over a finite period of time (Pearce and Kinsella, 1978). A higher EAI would be

an important attribute in choosing an effective protein emulsifier, since the higher the EAI, the smaller the amount of protein that would be required to stabilize the same amount of emulsion. Similar to CS, the EAI of faba bean protein isolate (13.0 m^2/g) was comparable to that of soy (14.6 m^2/g) but was much higher than that of pea protein isolate (1.7 m^2/g). Thus, faba bean protein isolates could be used in various food applications where emulsifying properties are essential especially in meat-based products. In contrast, the ESI of faba bean isolate (10.7 min) was lowest as compared to all of the commercial isolates (11.6-13.0 min). In general, except for ESI, the faba bean isolate had emulsifying properties similar to those of soy and whey protein isolates, and higher than that obtained for the pea protein isolate. Based on this study, the use of the pea protein isolate would not be recommended in emulsified food products due to its low CS and EAI.

With the exception of CS, values for all of the emulsifying properties (EC, EAI and ESI) for the faba bean protein isolates prepared in this study were much lower than those of another faba bean protein isolate prepared using an alkaline extraction/isoelectric precipitation process (513.3 g oil/ g protein, 44.3 m^2/g and 69.4 min, respectively) (Can Karaca et al., 2011). The higher value of EC obtained in the study of Can Karaca et al. (2011) was due to differences in the method of measuring EC where a higher amount of initial protein solution and a lower protein concentration was used. Similarly, the ECs of faba bean isolates produced in this study were lower than those of other faba bean, pea and soybean protein isolates (EC analysed using a different method) prepared by isoelectric precipitation (~354 g oil/g protein, ~335 g oil/g protein and ~413 g oil/g protein, respectively) (Sosulski and McCurdy, 1987). This demonstrates that comparing EC values from the literature is problematic because of the different methods and conditions employed for EC measurement, which gives quite varied values. However, despite the use of the same methods for measuring EAI and ESI as did Can Karaca et al. (2011), lower values were attained for faba bean isolate in the current sturdy; these differences may be due to the influence of feedstock composition.

Physicochemical parameters, including ZP, SH, solubility and IT, were reported by Can Karaca et al. (2011) to influence emulsifying properties. In contrast, with the exception of ZP, in the current study no physicochemical parameter (SH, solubility, IT) was found to be correlated with any of the emulsifying parameters studied. The CSs of faba bean isolates were found to be positively correlated with ZP ($r = 0.46$; $p<0.05$), in accordance with the study of Can Karaca et

al. (2011) where a positive correlation was observed between ZP and CS of a faba bean protein isolate ($r = 0.60$; $p<0.01$). This correlation suggests that a higher ZP, which results in strong electrostatic repulsion between protein molecules, would be an important attribute in emulsion stabilization. Furthermore, isolates prepared by an isoelectric precipitation method were reported to have a higher ZP as compared to those prepared by a salt extraction method (Can Karaca et al., 2011), which suggests the influential role of extraction method on emulsifying properties. However, there are other factors other than physicochemical properties which influence the ability of the proteins to bind to gas bubbles or oil droplets, which include the size, shape and conformation of proteins, nature of amino acids, lipid or carbohydrates attached to the protein surface, and thermodynamic properties (Kinsella, 1981; Sathe and Salunkhe, 1981; Ragab et al., 2004; Can Karaca et al., 2011; Joshi et al., 2012). Thus, future studies considering these factors would be essential in studying emulsifying properties at the structural/functional level.

5.4.3 Foaming capacity and stability

The mechanism of foam formation and stability is very similar to that of emulsion formation and stability. In a protein-based foam system, protein molecules adsorb at the air-water interfaces of air bubbles and aid in reducing surface tension. With more and more protein molecules adsorbing at the surface of air bubbles, protein molecules unravel and form an interfacial film, thus preventing bubble fusion (via Oswald ripening) or their collapse in the suspension for a certain period of time (Boye et al., 2010a; Kiosseoglou and Paraskevopoulou, 2011). The entrapment of air in the form of bubbles after homogenizing a protein solution is the key aspect in the formation of a number of food products such as meringues, whipped toppings, mousses and cake batters (Kiosseoglou and Paraskevopoulou, 2011). The foam properties of proteins are commonly evaluated as foaming capacity (FC) and foam stability (FS). Foaming capacity is a measure of the volume of foam generated after homogenization of a protein solution for a defined period of time, whereas FS is a measure of the decrease in foam volume of the same suspension after a certain period of time. In this study, the mean FC and FS values of the faba bean protein isolates (independent of genotype) were ~162% and ~65%, respectively. The FC of faba bean isolates was found to be similar in magnitude to those of soy (157%), pea (150%) and egg (112%) isolates ($p>0.05$), but lower than the FC of whey (228%) and wheat protein (223%) isolates ($p<0.01$). In contrast, the FS was similar among faba bean and all of the

commercial isolates ($p>0.05$).

Foaming properties (FC and FS) were independent of genotype, although differences due to environment were observed. In the literature, various studies have suggested that proteins with high solubility produce more foam, as they easily unfold and refold at the interface, thus reducing the interfacial tension between air and the protein surface (Chavan et al., 2001; Kaur and Singh, 2007; Adebiyi and Aluko, 2011). In accordance with this, faba bean protein isolates with higher foaming capacities were obtained in this study, due probably to their high solubility in aqueous solution; however, no significant correlation was observed with solubility or any of the functional or physicochemical parameters. In literature foam stability is found to be influenced by the structure of proteins, and dependent on electric charge and electrostatic repulsion between protein molecules (Chavan et al., 2001; Kaur and Singh, 2007; Adebiyi and Aluko, 2011). In contrast to what expected, foaming stability (FS) was found to be positively correlated with zeta potential (ZP) ($r = 0.54$; $p<0.01$). The FC of faba bean protein isolates was similar, and the FS slightly lower, than values reported for another faba bean protein isolate prepared using isoelectric precipitation (FC = ~160% and FS = ~77%) (Fernandez-Quintela et al., 1997). It was reported that the addition of NaCl increased the foaming capacity of lupin, pea and faba bean protein isolates, and there was a difference in foaming properties at pH 5.5 and 7.0 (Makri et al., 2005). This suggests that processing conditions (pH, temperature, salt concentration) play a significant role in determining the foaming properties of protein preparations.

5.4.4 Protein solubility

Solubility is an important attribute of proteins, and by manipulating solubility, protein isolates/concentrates with varying purities and functionalities can be obtained (Boye et al., 2010a; Kiosseoglou and Paraskevopoulou, 2011). A number of parameters, including foaming and emulsification, are dependent on the solubility of protein materials. For instance, a well dispersed protein solution has better emulsion-forming properties since protein molecules can rapidly migrate to oil/water interfaces, which leads to higher creaming stability (Sikorski, 2001). The solubility profile was found to be positively correlated with zeta potential (ZP) ($r = 0.44$; $p<0.05$) and negatively correlated with interfacial tension (IT) ($r = -0.38$; $p<0.05$) and oil holding capacity (OHC) ($r = -0.38$; $p<0.05$). A protein with higher ZP will lead to higher solubility by

providing greater electrostatic repulsion between molecules, thereby inhibiting aggregation (Can Karaca et al., 2011). In order for a protein to lower interfacial tension at an oil-water interface, it needs to have intermediate hydrophobicity and charge to allow it to integrate and re-align at the interface. Too little hydrophobicity would prevent it from integrating at the interface, whereas too much would reduce its solubility. At the same time, a highly charged protein would induce repulsive forces at the interface, inhibiting the formation of a viscoelastic film at the interface. Based on this, proteins with low surface hydrophobicity and high solubility would have difficulty lowering IT. Further, proteins with higher solubility tend to me more hydrophilic, suggesting that their ability to hold or bind oil would be less, resulting in a negative correlative relationship between solubility and OHC.

The average solubility, irrespective of genotype, of faba bean isolates (81%) was higher than those of the commercial isolates i.e., pea (~20%), wheat (~11%) and soy (31%) ($p<0.05$), but was comparable to those of commercial isolates from whey (89%) or egg (~81%) ($p>0.05$). However, the solubility of the faba bean isolate was lower than values obtained for another faba bean isolate (~90%) and other pea (~62%), soy (~97%), lentil (~91%), and chickpea (~91%) isolates prepared using isoelectric precipitation (Can Karaca et al., 2011). Differences in the solubility of various protein ingredients may be the result of different feed stocks, e.g., wheat proteins are dominated by alcohol soluble prolamins, or processing conditions. The latter plays a large role in terms of protein denaturation, which is associated with the breakage of hydrogen bonds and unravelling of the quaternary and tertiary structure of proteins and exposure of buried hydrophobic moieties. For instance, most commercial operations are presumed to use spray drying rather than freeze drying. Spray drying, although advantageous in terms of high throughput and low production cost, may adversely affect protein functionality since it utilizes a much higher temperature than does freeze drying. Otegui et al. (1997) reported that faba bean concentrates obtained by freeze drying had 38% higher solubility than those that were spray dried, where the inlet and outlet air temperatures of the spray dryer were 190°C and 122°C, respectively.

5.5 G x E analysis

G x E assessment was performed by analysing interactions between genotype and location (G x Y), and genotype and year (G x Y) for the SSNS-1, FB9-4 and FB18-20 genotypes

only. In general, the effect of year on all parameters was found to be less significant than the effect of location. Very few G x Y interactions were observed as compared to the number of G x L interactions. Most of the flour and isolate proximate components analyzed exhibited significant differences due to the individual and interactive effects of genotype and location. However, except for flour ash, a G x Y interaction was absent for all proximate components, and the effect of year was limited to faba bean flour and not isolates. This suggests that the year effect was not as prominent as location, and the variation due to year could be minimized by using modified protein extraction methodologies. A significant G x L interaction for physicochemical and functional properties was limited to OHC, FC, EC, Sol and SH, and in the case of G x Y, to EC. Interestingly, except for FC and ESI, the effect of year on all other physicochemical and functional properties was not significant. Differences in the colour characteristics of faba bean flours and isolates were profound due to the effects of location and genotype, where a G x L interaction and all the main effects had significant effects on all colour parameters. However, as observed for other properties, the effect of year on colour parameters was limited. The L/V of isolates was influenced only by location and had a significant G x L interaction, which suggests that although genotype did not have a direct effect on L/V ratio, it is an important factor, which along with location, can significantly influence the L/V ratio.

5.6 Applications

Pulse protein isolates and concentrates have potential for use as functional ingredients in the formulation of various food products (shakes, energy bars, beverages, snacks and meat analogues) or the fortification of existing ones. Food manufacturers rely on the desirable functional attributes of protein ingredients, such as their ability to emulsify, foam, solubilize etc. The protein ingredients market is growing rapidly, where protein ingredients such as those for pea, soy and whey are quite popular. Soy proteins already have been exploited commercially in formulating a wide variety of food products owing to their nutritional and functional properties. For instance, soy isolate and whey protein blends are used to make cakes, and may completely replaces non-fat dry milk (NFDM) without having adverse effects on quality (Turro and Sipos, 1970). The superior emulsifying properties of soy proteins provide uniform, smoother, pliable and less sticky characteristics to dough which results in improved crust colour and texture of bakery products (Endres, 2001). Along with dairy-based products, the use of soy proteins is also

increasing in processed meat systems where it is used as partial meat substitute, emulsifier, flavour enhancer and meat analogue. One of the important applications of soy proteins is reducing cooking loss and tenderization of whole muscle meat, which is done by injection of soy protein brine solution (Endres, 2001). In general, meat products containing soy protein provide excellent organoleptic and textural properties, and provide more yield per pound of meat (Kinsella et al., 1985; Kolar et al., 1985). However, Soderberg (2013) reported that products made from soy and pea proteins had lower acceptability than those made from egg proteins, which may be because of the beany and hay-like flavour of legume proteins.

Peazazz and CLARISOY, pea and soy protein isolates, respectively, produced by Burcon NutraScience (Vancouver, BC, Canada) and Archer Daniels Midland Company (Decatur, IL, USA) are reported to have 90% protein content, 100% solubility at acidic pH, neutral taste, heat stability and low viscosity. Their industrial uses are stated to be in the preparation of low pH beverages and fortified waters, and in a variety of food, beverage and nutritional applications. The faba bean protein isolates produced in this study had protein contents of more than 90%; however, their protein solubility was ~81%, which is less than that of CLARISOY and Peazazz products. The faba bean isolates were observed to have emulsification and foaming values comparable to those of soy, pea and egg isolates, which suggests promising applications in meat and dairy products such as sausages, frozen desserts and whipped creams (Hayat et al., 2014). Moreover, the high OHC values of the faba bean protein isolate in comparison to commercial isolates could provide it with a competitive advantage in food processing and product storage by helping in reducing cooking loss and fluid leakage, respectively. However, very few studies to date have examined faba bean proteins for their functionality and consumer acceptance. Cai et al. (2001) prepared a tofu-like product from protein extracts of soybean, faba bean and chickpea and found that faba bean and chickpea had comparable textural properties, but poorer than those of soybean. Sausage meat extenders fortified with 20% to 40% faba bean PMM (protein micellar mass) concentrates (prepared using salt extraction) were reported to be acceptable at all levels. In general, products made from faba bean PMM concentrates had more acceptability than faba bean flour fortified products (Abdel-Aal et al., 1987). However, despite of having suitable functionality and consumer acceptance, food products containing faba bean ingredients should be tested first for the presence of glycosides or other anti-nutritional factors before releasing into the market, or they otherwise could possess serious concerns with respect to food safety.

5.7 Factors affecting growth of faba bean and chemical composition

Despite the nutritional advantages of faba beans, there has been limited growth in production due to its high susceptibility to various biotic and abiotic stresses (Link et al., 2010; Sillero et al., 2010). For instance, broomrape species [(*Orobanche spp*. and *Phelipanche spp*.)] are weedy root parasites and are a serious concern in North Africa and sub Saharan-African countries where ~30% of the faba bean is produced (Maalouf, 2011). Faba bean also is very susceptible to heat waves and recurring droughts due to climatic variations, more so than other legumes (Maalouf, 2011). The International Center for Agricultural Research in the Dry Areas (ICARDA) (Beirut, Lebanon) is working on the development of new faba bean varieties and has screened and identified faba bean germplasm with resistance to abiotic (heat, cold and drought) and biotic stresses [leaf, stem and pod spot (*Ascochyta fabae*), chocolate spot (*Botrytis spp*.), rust (*Uromyces spp*.) and broomrape (*Orobanche spp*. and *Phelipanche spp*.)] (Khalil et al., 2004; Maalouf et al., 2010). The new germplasm developed at ICARDA is reported to have high yield, wide adaptability to different ecosystems, disease resistance, and high drought and heat tolerance.

Variation in the chemical composition of pulses (protein, ash, fibre, starch, etc.) has been attributed to environmental factors such as temperature, rainfall, lighting conditions and growing season (Singh et al., 1990; Nikolopoulou et al., 2006; Nikolopoulou et al., 2007). For instance, ~9% variability in the protein levels of winter-sown and spring-sown cultivars of faba bean was reported, which suggests the influence of climatic conditions (temperature and rainfall) during those seasons (Eden et al., 1968). Several cultivars of faba bean grown in Manitoba and Saskatchewan in 1970 and 1971 were reported to have protein content ranging from 23% to 32%; however, only minor variations because of location and year were observed (Evans et al., 1972). Similarly, Hood-Niefer et al. (2011) reported substantial differences in protein and starch concentrations of ten pea and eleven faba bean genotypes; however, differences due to environment (location and year) were reported to be minimal. In contrast, six pea cultivars grown in 2006 and 2007 at five locations in Saskatchewan showed significant differences in crude protein, starch, crude fibre, fat, ash and phytic acid due to the effect of bot cultivar and growing season and location (Wang et al., 2010). Differences in protein and starch levels were observed for faba bean cultivars grown in the summer of 1972 on University of Saskatchewan experimental plots; ranges were 26-35% and 28-41%, respectively. However, fibre and ash

contents were relatively uniform (Bhatty et al., 1974), suggesting that certain components are influenced more than others by environmental factors.

6. CONCLUSIONS AND FUTURE STUDIES

The overall aim of this research was to investigate the effect of genotype and the growth environment on the physicochemical and functional properties of the faba bean prepared isolates. Overall, genotype did not have a substantial impact on the functionality of the faba bean protein isolates with respect to any of the functional properties. Similarly, no differences in physicochemical properties were observed among faba bean genotypes. Variability within each genotype was observed for almost all tests measured in response to the environmental conditions, highlighting the complex relationship between plant biology and protein profiles. A G x E assessment was conducted for SSNS-1, FB9-4 and FB18-20 only, since they were grown at the same locations in 2011 and 2012 whereas PCA analysis was performed considering all seven genotypes grown at several locations in 2011 and 2012. A G x E assessment and PCA scatter plot showed the effect of location was more prominent than the effect of year. And, in PCA analysis zero tannin genotypes were found to be grouped together and opposite to those of tannin-containing genotypes. A mean protein content of ~94% and an isolate yield of ~25% were achieved for faba bean protein isolates. Although it was originally hypothesized originally that the legumin:vicilin (L/V) ratio would significantly impact protein functionality, and would differ depending on the genotype, this was not found to be the case. Instead, results suggest that processing (i.e., the extraction method used to prepare the isolate) may play a greater role than biology, as it may select for one protein fraction over another depending on the processing conditions employed. Further investigation of optimizing the L/V ratio in response to extraction conditions is warranted.

The functional properties of faba bean isolates were found to be comparable to those of some of the commercial isolates tested in this study. In general, with the exception of creaming stability (CS) for the pea and soy isolates and foaming capacity (FC) for the egg isolate, the foaming and emulsifying properties of pea, egg and soy were found to be similar to those of faba bean isolates. The whey protein isolate was found to have similar foam stability (FS), CS, emulsion activity index (EAI) values, and higher FC, emulsion capacity (EC) and emulsion stability index (ESI) values as compared to faba bean protein isolates, whereas the wheat protein

isolate had comparable foaming properties but lower emulsifying properties. The oil holding capacity (OHC) of faba bean isolates was observed to be significantly higher than that of any of the commercial isolates, and with the exception of whey and egg isolates, the solubility of commercial isolates was found to be similar in magnitude to that of faba bean protein isolates. These results suggest that faba bean isolates have good potential as alternative plant protein ingredients in the market. Furthermore, isolates obtained in this study, along with high protein content, contained negligible crude fat, and could be used in producing novel food products in the form of cookies, muffins, and protein bars. Similarly, isolates with better emulsifying properties would be beneficial in producing a wide variety of meat-based products such as sausages and bologna. However, despite all these advantages, the integration of faba bean protein isolates into the current market is still limited because of the supply-demand gap in the production of faba beans, and food safety concerns associated with the consumption of raw faba bean. Moreover, extensive research is still required in exploring and controlling extraneous factors which could be limiting factors in producing stable genotypes.

Further research in this area could include:

a) Conducting a full genotype x environment study over several years with a larger number of genotypes. Locations could be broadened to different soil zones within the same geographic area (e.g., Canada), or taken from international locations. The information obtained would enable a stronger data set to be developed to better assess structure-function relationships within faba bean protein isolates.

b) Studying structure-dynamic-function relationships by examining protein kinetics under different conditions within solution, protein conformation and flexibility, and diffusion within the aqueous phase and at the oil-water and/or air-water interfaces.

c) Studying 2-D electrophoresis patterns of faba bean to identify the different polypeptides comprising legumin and vicilin, since all have different molecular weights and isoelectric points (Tucci et al., 1991). These polypeptides could be studied in relation to differences in the legumin:vicilin ratio, protein content and functional/physicochemical properties. The information obtained would aid in undertanding structure-function relationships at the molecular level, and with the involvement of extrinsic factors it could be assessed whether differences are due to intrinsic (genetic origin) or extrinsic (pH, ionic, extraction method, climatic conditions, etc.) factors.

d) Conducting product development studies where faba bean isolates were used as one of the major ingredients and compared with established commercial ingredients in the market. Important parameters which could be looked at evaluated would include texture, consistency, organoleptic attributes, shelf life and safety of the product. Most importantly, human consumption trials of faba bean based products are required to provide safety with respect to favism or other antinutrient based toxicity.

7. REFERENCES CITED

Abdel-Aal, E. M., Youssef, M. M., Shehata, A. A., & El-Mahdy, A. R. (1987). Some legume proteins as bread fortifier and meat extender. *Alexandria Journal of Agricultural Research, 32*, 179-189.

Adebiyi, A. P., & Aluko, R. E. (2011). Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum sativum* L.) seed protein isolate. *Food Chemistry, 128*, 902-908.

Adebowale, Y. A., Adeyemi, I. A., & Oshodi, A. A. (2005). Variability in the physicochemical, nutritional and antinutritional attributes of six *Mucuna* species. *Food Chemistry, 89*, 37-48.

Adebowale, Y. A., Adeyemi, I. A., Oshodi, A. A., & Niranjan, K. (2007). Isolation, fractionation and characterisation of proteins from Mucuna bean. *Food Chemistry, 104*, 287-299.

Aguilera, J. M., & Garcia, H. D. (1989). Protein extraction from lupin seeds: a mathematical model. *International Journal of Food Science and Technology, 24*, 17-27.

Aguilera, Y., Diaz, M. F., Jimenez, T., Benitez, V., Herrera, T., Cuadrado, C., Martin-Pedrosa, M., & Martin-Cabrejas, M. A. (2013). Changes in nonnutritional factors and antioxidant activity during germination of nonconventional legumes. *Journal of Agricultural and Food Chemistry, 61*, 8120-8125.

Alamanou, S., Blouksa, J. G., Paneras, E. D., & Doxastakis, G. (1996). Influence of protein from lupin seeds (*Lupinus albus ssp. Graecus*) on processing and quality characteristics of frankfurters. *Meat Science, 42*, 79-93.

Alonso, R., Aguirre, A., & Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chemistry, 68*, 159-165.

Alsohaimy, S. A., Sitohy, M. R., & El-Masry, R. A. (2007). Isolation and partial characterization of chickpea, lupine and lentil seed proteins. *World Journal of Agriculture Sciences, 3*, 123-129.

Amarteifio, J. O., Munthali, D. C., Karikari, S. K., & Morake, T. K. (2002). The composition of pigeon pea (*Cajanus cajan* (L) Millsp) grown in Botswana. *Plant Foods for Human Nutrition, 57,* 173-177.

Andersson, O., Schmanke, H., Polyakov, V. I., Grinberg, V. Ya., Bikbov, T. M., Danilenko, A. P., Leontjev, A. L., & Tolstoguzov, V. B. (1985). Compatibility of gelation with broad bean globulin in an aqueous medium. *Journal of Food Science, 50,* 1133-1135.

Anonynous (n.d.) What are pulses? In *Food and Nutrition (Manitoba Pulse Growers Association Inc.)*. Retrieved from http://www.manitobapulse.ca/for-consumers/what-are-pulses/

AOAC (2003). *Official Methods of Analysis of AOAC International, 17th Edition, Revision 2*. Gaithersburg: Association of Official Analytical Chemists, Inc.

Avramenko, N. A., Low, N. H., & Nickerson, M. T. (2013). The effects of limited enzymatic hydrolysis on the physicochemical and emulsifying properties of a lentil protein isolate. *Food Research International, 51,* 162-169.

Barac, M., Cabrilo, S., Pesic, M., Stanojevic, S., Zilic, S., Macej, O., & Ristic, N. (2010). Profile and functional properties of seed proteins from six pea (*Pisum sativum*) genotypes. *International Journal of Molecular Sciences, 11,* 4973-4990.

Benitez, V., Cantera, S., Aguilera, Y., Molla, E., Esteban, R. M., Diaz, M. F., & Martin-Cabrejas, M. A. (2013). Impact of germination on starch, dietary fibre and physicochemical properties in nonconventional legumes. *Food Research International, 50,* 64-69.

Bhatty, R. S. (1982). Albumin proteins of eight edible grain legume species: Electrophoresis patterns and amino acid composition. *Journal of Agricultural and Food Chemistry, 30,* 620-622.

Black, R. G., Brouwer, J. B., Meares, C., & Iyer, L. (1998). Variation in physicochemical properties of field peas (*Pisum sativum*). *Food Research International, 31,* 81-86.

Bora, P. S., Brekke, C. J., & Powers, J. R. (1994). Heat induced gelation of pea (*Pisum sativum*) mixed globulins, vicilin and legumin. *Journal of Food Science, 59,* 594-596.

Boye, J., Zare, F., & Pletch, A. (2010a). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International, 43,* 414-431.

Boye, J. I., Akshay, S., Ribereau, S., Mondor, M., Farnworth, E., & Rajamohamed, S. H. (2010b). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International, 43*, 537-546.

Brewer, M. S. (2012). Reducing the fat content in ground beef without sacrificing quality: A review. *Meat Science, 91*, 385-395.

Cai, R., Klamczynska, B., & Baik, B. K. (2001). Preparation of bean curds from protein fractions of six legumes. *Journal of Agricultural and Food Chemistry, 49*, 3068-3073.

Cai, T., & Chang, K. C. (1999). Processing effect on soybean storage proteins and their relationship with tofu quality. *Journal of the Science of Food and Agriculture, 47*, 720-727.

Campbell, L. D., Olaboro, G., Marquardt, R. R., & Waddell, D. (1980). Use of faba beans in diets for laying hens. *Canadian Journal of Animal Science, 60*, 395-405.

Can Karaca, A., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International, 44*, 2742-2750.

Casey, R., Sharman, J. E., Wright, D. J., Bacon, J. R., & Guldager, P. (1982). Quantitative variability in *Pisum* seed globulins: Its assessment and significance. *Plant Foods for Human Nutrition, 31*, 333-346.

Cepeda, E., Villaran, M. C., & Aranguiz, N. (1998). Functional properties of faba bean (*Vicia faba*) protein flour dried by spray drying and freeze drying. *Journal of Food Engineering, 36*, 303-310.

Cerning, J., Saposnik, A., & Guilbot, A. (1975). Carbohydrate composition of horse beans (*Vicia faba*) of different origins. *Cereal Chemistry, 52*, 125-137.

Chakraborty, P. (1986). Coconut protein isolate by ultrafiltration. In M. LeMeguer, & P. Jelen (Eds.), *Food Engineering and Process Applications* (pp. 308-315). New York: Elsevier Applied Science Publishers.

Chavan, U. D., McKenzie, D. B., & Shahidi, F. (2001). Functional properties of protein isolates from beach pea (*Lathyrus maritimus* L.). *Food Chemistry, 74*, 177-187.

Coda, R., Melama, L., Rizzello, C. G., Curiel, J. A., Sibakov, J., Holopainen, U., Pulkkinen, M., & Sozer, N. (2015). Effect of air classification and fermentation by *Lactobacillus plantarum* VTT E-133328 on faba bean (*Vicia faba* L.) flour nutritional properties. *International Journal of Food Microbiology, 193*, 34-42.

Cousin, R. (1997). Peas (*Pisum sativum* L.). *Field Crops Research, 53*, 111-130.

Crepon, K., Marget, P., Peyronnet, C., Carrouee, B., Arese, P., & Duc, G. (2010). Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. *Field Crop Research, 115*, 329-339.

Croy, R. R., Gatehouse, J. A., Tyler, M., & Boulter, D. (1980). The purification and characterization of a third storage protein (convicilin) from the seeds of pea (*Pisum sativum* L.). *Biochemical Journal, 191*, 509-516.

Cserhalmi, Z., Czukor, B., & Gajzago-Schuster, I. (1998). Emulsifying properties, surface hydrophobicity and thermal denaturation of pea protein fractions. *Acta Alimentaria, 27*, 357-363.

Damiani, E., Aloia, A. M., Priore, M. G., Pastore, A., Nardulli, S., & Lippolis, C. (2011). *Vicia faba* hypersensitivity and ASA intolerance in a farmer: a case report. *Journal of Allergy, 11*, 1-4.

Damodaran, S. (2005). Protein stabilization of emulsions and foams. *Journal of Food Science, 70*, 54-66.

Danielsson, C. E. (1949). Seed globulins of gramineae and leguminosae. *Biochemical Journal, 44*, 387-400.

Davidson, J. (1980). Attempts to eliminate from field beans the anti-nutritive factor which reduces egg production. *British Poultry Science, 21*, 299-307.

Deliza, R., Saldivar, S. O. S., Germani, R., Benassi, V. T., & Cabral, L. C. (2002). The effects of coloured textured soybean protein (TSP) on sensory and physical attributes of ground beef patties. *Journal of Sensory Studies, 17*, 121-132.

Derbyshire, E., Wright, D. J., & Boulter, D. (1976). Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry, 15*, 3-24.

Dervas, G., Doxastakis, G., Hadjisavva-Zinoviadi, S., & Triantafillakos, N. (1999). Lupin flour addition to wheat flour doughs and effect on rheological properties. *Food Chemistry, 66*, 67-73.

Desnoyers, J. E., & Jolicoeur, C. (1969). Hydration effects and thermodynamics properties of ions. In J. O'M. Bockris, & B. E. Conway (Eds.), *Modern Aspects of Electrochemistry No.5*. New York: Plenum Press.

Duc, G., Bao, S. Y., Baum, M., Redden, B., Sadiki, M., Suso, M. J., Vishniakova, M., & Zong, X. X. (2010). Diversity maintenance and use of *Vicia faba* L. genetic resources. *Field Crops Research, 115*, 270-278.

Duc, G., Marget, P., & Arese, P. (2011). Breeding priorities for improved nutritional value of *Vicia faba* seeds. *Grain Legumes, 56*, 16-18.

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. *Fitoterapia, 77*, 67-82.

Eden, A. (1968). A survey of the analytical composition of field beans (*Vicia faba* L.). *Journal of Agricultural Science, 70*, 299-301.

El fiel, H. E. A., El Tinay, A. H., & El Sheikh, E. A. E. (2002). Effect of nutritional status of faba bean on protein solubility profiles. *Food Chemistry, 76*, 216-223.

Endres, J. G. (2001). Soy protein products: characteristics, nutritional aspects, and utilization. AOCS Press (pp. 31-40). Available (electronic) *http://enerex.s3.amazonaws.com/articles/soy_protein_products.pdf*.

Escamilla-Silva, E. M., Guzman-Maldonado, S. H., Cano-Medinal, A., & Gonzalez-Alatorre, G. (2003). Simplified process for the production of sesame protein concentrate. Differential scanning calorimetry and nutritional, physicochemical and functional properties. *Journal of the Science of Food and Agriculture, 83*, 972-979.

Eucken, A., & Hertzberg, G. (1950). Aussalzeffekte Und Ionehydratation. *Angewandte Chemie, 62*, 196-196

Evans, L. E., Seitzer, J. F., & Buskuk, W. (1972). Horsebeans: A protein crop for Western Canada. *Canadian Journal of Plant Science, 52*, 657-659.

Fan, T. Y., & Sosulski, F. W. (1974). Dispersibility and isolation of proteins from legume flours. *Canadian Institute of Food Science Technology, 7*, 256-259.

Fernandez-Quintela, A., Macarulla, M. T., Del Barrio, A. S., & Martinez, J. A. (1997). Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. *Plant Foods for Human Nutrition, 51*, 331-342.

Flink, J., & Christiansen, I. (1973). The production of protein isolate from *Vicia faba*. *Food Science and Technology, 6*, 102-106.

Foegeding, E. A., & Davis, J. P. (2011). Food protein functionality: A comprehensive approach. *Food Hydrocolloids, 25*, 1853-1864.

Frank-Peterside, N., Dosumu, D. O., & Njoku, H. O. (2002). Sensory evaluation and proximate analysis of African yam bean (*Strepnostylis stenocarpa*). *Journal of Applied Science and Environmental Management, 6*, 43-48.

Fredrikson, M., Biot, P., Alminger, M. L., Carlsson, N. G., & Sandberg, A. S. (2001). Production process for high quality pea protein isolate with low content of oligosaccharides and phytate. *Journal of Agricultural and Food Chemistry, 49*, 1208-1212.

Frias, J., DiazPollan, C., Hedley, C. L., & Vidal-Valverde, C. (1995). Evolution of trypsin inhibitor activity during germination of lentils. *Journal of Agriculture and Food Chemistry, 43*, 2231-2234.

Fuhrmeister, H., & Meuser, F. (2003). Impact of processing on functional properties of protein products from wrinkled peas. *Journal of Food Engineering, 56*, 119-129.

Galazka, V. B., Dickinson, E., & Ledward, D. A. (1999). Emulsifying behavior of 11S globulin *Vicia faba* in mixtures with sulphated polysaccharides: comparison of thermal and high-pressure treatments. *Food Hydrocolloids, 13*, 425-435.

Gatehouse, J. A., Croy, R. R. D., Mclntosh, R., Paul, C., & Boulter, D. (1980). Quantitative and qualitative variation in the storage proteins of material from the EEC point field bean test. In D. A. Bond (Ed.), *Vicia faba: Feeding Value, Processing and Viruses* (pp. 173-190). Boston: Martinus Nuhoff Publishers.

Glasstone, S., Dimond, D. W., & Jones, E. C. (1926). Solubility influences. Part II. The effect of various salts on the solubility of ethyl acetate in water. *Journal of the Chemical Society, 129*, 2935-2939.

Glasstone, S., Bridgman, J., & Hodgson, W. R. P. (1927). Solubility influences. Part IV. The salting-out of aniline from aqueous solutions. *Journal of the Chemical Society, 0*, 635-640.

Granito, M., Frias, J., Doblado, R., Guerra, M., Champ, M., & Vidal-Valverde, C. (2002). Nutritional improvement of beans (*Phaseolus vulgaris* L.) by natural fermentation. *European Food Research and Technology, 214*, 226-231.

Grover, P. K., & Ryall, R. L. (2005). Critical appraisal of salting-out and its implications for chemical and biological sciences, *Chemical Reviews, 105*, 1-10.

Guan, X., Yao, H., Chen, Z., Shan, L., & Zhang, M. (2007). Some functional properties of oat bran protein concentrate modified by trypsin. *Food Chemistry, 101*, 163-170.

Gueguen, J. (1983). Legume seed protein extraction, processing, and end product characteristics. *Plant Foods for Human Nutrition, 32*, 267-303.

Gueguen, J., & Barbot, J. (1988). Quantitative and qualitative variability of pea (*Pisum sativum* L.) protein composition. *Journal of the Science of Food and Agriculture, 42*, 209-224.

Gueguen, J., & Cerleti, P. (1994). Proteins of some legume seeds: soybean, pea, faba bean and lupin. In B. J. F. Hudson (Ed.), *New and Developing Sources of Food Proteins* (pp. 145-193). London: Chapman and Hall.

Gupta, R., & Dhillon, S. (1993). Characterization of seed storage proteins of lentil (*Lens culinaris* M.). *Annals of Biology, 9*, 71-78.

Gwiazda, S., Schwenke, K. D., & Rutkowski, A. (1980). Isolation and partial characterization of protein from pea. *Nahrung, 24*, 939-950.

Hajoos, G., & Osagie, A. U. (2004). Technical and biotechnological modifications of antinutritional factors in legumes and oilseeds. *Proceedings of the Fourth International Workshop on Antinutritional Factors in Legume Seeds and Oilseeds*, 293-305.

Han, J., & Khan, K. (1990). Physicochemical studies of pin-milled and air-classified dry edible bean fractions. *Cereal Chemistry, 67*, 384-390.

Han, J., Hanz, J. A. M., & Gerlat, M. (2010). Development of gluten-free cracker snacks using pulse flours and fractions. *Food Research International, 43*, 627-633.

Han, Z., & Hamaker, B. R. (2002). Partial leaching of granule-associated proteins from rice starch during alkaline extraction and subsequent gelatinization. *Starch–Starke, 54*, 454-460.

Hayat, I., Ahmad, A., Ahmed, E., Khalil, S., & Gulfraz, M. (2014). Exploring the potential of red kidney beans (*Phaeolus vulgaris* L.) to develop protein based product for food applications. *The Journal of Animal & Plant Sciences, 24*, 860-868.

Hill, S. E. (1996). Emulsions. In G. M. Hall (Ed.), *Methods of Testing Protein Functionality* (pp. 153-185). London: Blackie Academic & Professional.

Hofmeister, F. (1888). About the science of the effect of salts. *Archive for Experimental Pathology and Pharmacology, 24*, 247-260.

Hood-Niefer, S. D., Warkentin, T. D., Chibbar, R. N., Vandenberg, A., & Tyler, R. T. (2011). Effect of genotype and environment on the concentrations of starch and protein in, and the physicochemical properties of starch from, field pea and fababean. *Journal of the Science of Food and Agriculture, 92*, 141-150.

Jezierny, D., Mosenthin, R., & Bauer, E. (2010). The use of grain legumes as a protein source in pig nutrition: a review. *Animal Feed Science and Technology, 157*, 111-128.

Joshi, M., Adhikari, B., Aldred, P., Panozzo, J. F., Kasapis, S., & Barrow, C. J. (2012). Interfacial and emulsifying properties of lentil protein isolate. *Food Chemistry, 134*, 1343-1353.

Jung, S., Murphy, P. A., & Johnson, L. A. (2005). Physicochemical and functional properties of soy protein substrates modified by low levels of protease hydrolysis. *Journal of Food Science, Food Chemistry and Toxicology, 70*, 180-187.

Kaur, M., & Singh, N. (2007). Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. *Food Chemistry, 102*, 366-374.

Khalil, S., Kharrat, M., Malhotra, R. S., Saxena, M. C., & Erskine, W. (2004). Breeding faba bean for *Orobanche* resistance. Proceedings in *Expert Consultation on IPM for Orobanche in Food Legume Systems in the Near East and North Africa* (pp. 1-18). Rabat, Morocco.

Kim, Y. H., Kim, S. D., & Hong, E. H. (1994). 11S and 7S globulin fractions in soybean seed and soycurd characteristics. *Korean Journal of Crop Science, 39*, 348-352.

Kimura, A., Fukuda, T., Zhang, M., Motoyama, S., Maruyama, N., & Utsumi, S. (2008). Comparison of physicochemical properties of 7S and 11S globulins from pea, fava bean, cowpea, and French bean with those of soybean - French bean 7S globulin exhibits excellent properties. *Journal of Agriculture and Food Chemistry, 56*, 10273-10279.

Kinsella, J. E. (1979). Functional properties of soy proteins. *Journal of the American Oil Chemist's Society, 56*, 242-258.

Kinsella, J. E. (1981). Functional properties of proteins: Possible relationships between structure and function in foams. *Food Chemistry, 7*, 273-288.

Kinsella, J. E., Damodaran, S., & German, B. (1985). Physicochemical and functional properties of oilseed proteins with emphasis on soy proteins. In A. M. Alschul, & H. L. Wilcke (Eds.), *New Protein Foods: Seed Storage Proteins*, vol. 5 (pp. 107-179). New York: Academic Press.

Kiosseoglou, V., & Paraskevopoulou, A. (2011). Functional and physicochemical properties of pulse proteins. In B. K. Tiwari, A. Gowen, & B. Mckenna (Ed.), *Pulse Foods Processing: Quality & Nutritional Applications* (pp. 57-89). San Diego: Academic Press.

Klassen, D. R., & Nickerson, M. T. (2012). Effect of pH on the formation of electrostatic complexes within admixtures of partially purified pea proteins (legumin and vicilin) and gum arabic polysaccharides. *Food Research International, 46*, 167-176.

Knopfe, C., Schwenke, K. D., Mothes, R., Mikheeva, L. M., Grinberg, V. Ya., Gornitz, E., & Dautzenberg, H. (1998). Acetylation and succinylation of faba bean legumin: modification of hydrophobicity and conformation. *Nahrung, 42*, 194-196.

Kolar, C. W., Richert, S. H., Decker, C. D., Steinke, F. H., & Vander Zanden, R. J. (1985). Isolated soy protein. In A. A. Altschul, & H. L. Wilcke (Ed.), *New Protein Foods: Seed Storage Proteins*, vol. 5 (pp. 260-297). Orlando: Academic Press.

Kopke, U., & Nemecek, T. (2010). Ecological services of faba bean. *Field Crops Research, 115*, 217-233.

Koros, W. J., MA, Y. H., & Shimdzu, T. (1996). Terminology for membranes and membrane processes. *Pure & Applied Chemistry*, *68*, 1479-1489.

Kothekar, V. S., Harsulkar, A. M., & Khandelwal, A. R. (1996). Low trypsin and chymotrypsin inhibitor mutants in winged bean (*Psophocorpus tetragonolobus* L.*). Journal of the Science of Food and Agriculture, 71*, 137-140.

Kotula, A. W., & Berry, B. W. (1986). Addition of soy proteins to meat products. Plant Proteins: Applications, Biological Effects, and Chemistry. *American Chemical Society symposium series, 312*, 74-89.

Koyoro, H., & Powers, J. R. (1987). Functional properties of pea globulin fractions. *Cereal Chemistry, 64*, 97-101.

Kozlowska, H., Honke, J., Sadowska, J., Frias, J., & Vidal-Valverde, C. (1996). Natural fermentation of lentils: influence of time, concentration and temperature on the kinetics of hydrolysis of inositol phosphates. *Journal of the Science of Food and Agriculture, 71*, 367-375.

Kumar, D., Kumar, S., Verma, A. K., Sharma, A., Tripathi, A., Chaudhari, B. P., Kant, S., Das, M., Jain, S. K., & Dwivedi, P. D. (2014). Hypersensitivity linked to exposure of broad bean protein(s) in allergic patients and BALB/c mice. *Nutrition, 30*, 903-914.

Laemmli, U. K. (1970). Glycine-SDS-PAGE for separation of proteins. *Nature, 227*, 680-685.

Lampart-Szczapa, E. (2012). Legume and oilseed proteins. In Z. E. Sikorski (Ed.), *Chemical and Functional Properties of Food Proteins* (pp. 407-436). Lancaster, Basel: Technomic Publishing Co.

Lecomte, N., Zayas, J., & Kastner, C. (1993). Soy proteins functional and sensory characteristics improved in comminuted meals. *Journal of Food Science, 58*, 464-466.

Leyva-Lopez, N. E., Vasco, N., Barba de la Rosa, A. P., & Paredes-Lopez, O. (1995). Amaranth seed proteins: Effect of defatting on extraction yield and on electrophoretic patterns. *Plant Foods for Human Nutrition, 47*, 49-53.

Liener, I. E. (1990). Naturally occurring toxic factors in animal feedstuffs. In J. Wiseman, & D. J. A. Cole (Ed.), *Feedstuff Evaluation* (pp. 377-394). Guildford: Butterworths.

Link, W., Balko, C., & Stoddard, F. L. (2010). Winter hardiness in faba bean: physiology and breeding. *Field Crop Research, 115*, 287-296.

Liu, S., Elmer, C., Low, N. H., & Nickerson, M. T. (2010). Effect of pH on the functional behaviour of pea protein isolate-gum arabic complexes. *Food Research International, 43*, 489-495.

Luo, Y. W., Gu, Z. X., Han, Y. B., & Chen, Z. G. (2009). The impact of processing on phytic acid, in vitro soluble iron and Phy/Fe molar ratio of faba bean (*Vicia faba* L.). *Journal of the Science of Food and Agriculture, 89*, 861-866.

Maalouf, F. (2011). Fababean improvement at ICARDA: Constraints and challenges. *Grain Legumes, 56*, 13-14.

Maalouf, F., Ahmed, S., Kabakebji, M., Khalil, S., Abang, M., Kabbabeh, M., & Street, S. (2010). Sources of multiple resistances for key foliar diseases of faba bean. Proceedings, *5th International Food Legumes Research Conference and 7th European Conference on Grain Legumes* (pp.120). Antalya, Turkey.

Makri, E. A., Papalamprou, E. M., & Doxastakis, G. I. (2005). Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, pea, broad bean) in admixture with polysaccharides. *Food Hydrocolloids, 19*, 583-594.

Makri, E. A., Papalamprou, E. M., & Doxastakis, G. I. (2006). Textural properties of legume protein isolate and polysaccharide gels. *Journal of the Science of Food and Agriculture, 86*, 1855-1862.

Malav, O. P., Talukder, S., Gokulakrishnan, P., & Chand, S. (2015). Meat analogue: a review. *Critical Reviews in Food Science and Nutrition, 55*, 1241-1245.

Martensson, P. (1980). Variation in legumin:vicilin ratio between and within cultivars of *Vicia faba* L. var. minor. In D. A. Bond (Ed.), *Vicia faba: Feeding Value, Processing and Viruses* (pp. 159-168). Boston: Martinus Nuhoff Publishers.

Martin-Cabrejas, M. A., Diaz, M. F., Aguilera, Y., Benitez, V., Molla, E., & Esteban, R. M. (2008). Influence of germination on the soluble carbohydrates and dietary fibre fractions in nonconventional legumes. *Food Chemistry, 107*, 1045-1052.

McClements, D. J. (2005). *Food Emulsions: Principles, Practices, and Techniques.* Boca Raton: CRC Press.

McClements, D. J. (2007). Critical review of techniques and methodologies for characterization of emulsion stability. *Critical Reviews in Food Science and Nutrition, 47*, 611-649.

McCurdy, S., & Knipfel, J. (1990). Investigation of faba bean protein recovery and application to pilot scale processing. *Journal of Food Science, 55*, 1093-1094.

McMillan, D. C., Bolchoz, L. J. C., & Jollow, J. (2001). Favism: Effect of divicine on rat erythrocyte sulfhydryl status, hexose monophosphate shunt activity, morphology, and membrane skeletal proteins. *Toxicological Sciences, 62*, 353-359.

Mertens, C., Dehon, L., Bourgeois, A., Verhaeghe-Cartryssec, C., & Bleckera, C. (2011). Agronomical factors influencing the legumin/vicilin ratio in pea (*Pisum sativum* L.) seeds. *Journal of the Science of Food and Agriculture, 92*, 1591-1596.

Micek, P., Kowalski, Z. M., Kulig, B., Kanski, J., & Slota, K. (2015). The effect of variety and plant protection method on chemical composition and in vitro digestibility of faba bean (*Vicia faba*) seeds. *Annals of Animal Science, 15*, 143-154.

Mondor, M., Aksay, S., Drolet, H., Roufik, S., Farnworth, E., & Boye, J. I. (2009). Influence of processing on composition and anti-nutritional factors of chick pea protein concentrates produced by isoelectric precipitation and ultrafiltration. *Innovative Food Science & Emerging Technologies, 10*, 342-347.

Mori, T., & Utsumi, S. (1978). Purification and properties of storage proteins of broad bean. *Agriculture and Biological Chemistry, 43*, 577-583.

Mudryj, A. N., Yu, N., & Aukema, H. M. (2014). Nutritional and health benefits of pulses. *Applied Physiology Nutrition and Metabolism, 39*, 1197-1204.

Mujoo, R., Trinh, D. T., & Ng, P. K. W. (2003). Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. *Food Chemistry, 82*, 265-273.

Muller, G. G., & Redden, V. R. (1995). Sensory and functional evaluation of ground beef patties extended with milling grade culinary beans. *Proceedings of the Joint Australia and New Zealand Institute of Food Science and Technology Conference, 52*, 15-19.

Mur-Gimeno, P., Feo-Brito, F., Martin-Iglesias, A., Lombardero Vega, M., & Bautista-Martinez, P. (2007). Allergic reaction caused by a new hidden food, broad bean flour. *European Journal of Allergy and Clinical Immunology, 62*, 1340-1341.

Murphy, P. A., & Resurreccion, A. P. (1984). Varietal and environmental differences in soybean glycinin and β-conglycinin content. *Journal of Agricultural and Food Chemistry, 32*, 911-915.

Nakamura, T., Utsumi, S., & Mori, T. (1986). Mechanism of heat-induced gelation and gel properties of soybean 7S-globulin. *Agricultural and Biological Chemistry, 50*, 1287-1293.

Nikolic, Z., Dorpevic, V., Torbica, A., & Mikic, A. (2012). Legumes seed storage proteins characterization by SDS-PAGE and Lab-on-a-Chip electrophoresis. *Journal of Food Composition and Analysis, 28*, 25-80.

Nikolopoulou, D., Grigorakis, K., Stasini, M., Alexis, M. N., & Iliadis, K. (2006). Effects of cultivation area and year on proximate composition and anti-nutrients in three different Kabuli-type chickpea (*cicer arientium*) varieties. *European Food Research and Technology, 223*, 737-741.

Nikolopoulou, D., Grigorakis, K., Stasini, M., Alexis, M. N., & Iliadis, K. (2007). Differences in chemical composition of field pea (*Pisum sativum*) cultivars: Effect of cultivation area and year. *Food Chemistry, 103*, 847-852.

Nostro, P. Lo., & Ninham, B. W. (2012). Hofmeister phenomena: an update on ion specificity in biology, *Chemical Reviews, 112*, 2286-2322.

O'Kane, F. E., Happe, R. P., Vereijken, J. M., Gruppen, H., & Van Boekel, M. A. J. S. (2004). Characterization of pea vicilin. 1. Denoting convicilin as the r-subunit of the *Pisum* vicilin family. *Journal of Agriculture and Food Chemistry, 52*, 3141-3148.

Ofuya, Z. M. (2002). The ability of raw and processed cowpeas to support growth in weanling wistar strain rats. *Global Journal of Pure and Applied Sciences, 8*, 499-504.

Ofuya, Z. M., & Akhidue, V. (2005). The role of pulses in human nutrition: A review. *Journal of Apply Science and Environmental Management, 9*, 99-104.

Oke, D. B., Tewe, O. O., & Fetuga, S. L. (1995). The nutrient composition of some cowpea varieties. *Nigerian Journal of Animal Production, 22*, 32-35.

Onigbinde, A. O., & Onobun, V. (1993). Effect of pH on some cooking properties of cowpea (*V. unguiculata*). *Food Chemistry, 47*, 125-127.

Oomah, B. D., Patras, A., Rawson, A., Singh, N., & Compos-Vega, R. (2011). Chemistry of Pulses. In B. K. Tiwari, A. Gowen, & B. Mckenna (Eds.), *Pulse Foods Processing: Quality & Nutritional Applications* (pp. 9-55). San Diego: Academic Press.

Osborne, T. B. (1924). The vegetable proteins. *Monographs in Biochemistry*. London: Longmans, Green and Company.

Otegui, I., Fedndez-Quintela, A., De Diego, A., Cid, C., Macarullam, M. T., & Partearroyo, M. A. (1997). Properties of spray-dried and freeze-dried faba bean protein concentrates. *International Journal of Food Science and Technology, 32*, 439-443.

Panyam, D., & Kilara, A. (1996). Enhancing the functionality of food proteins by enzymatic modification. *Trends in Food Science & Technology, 7*, 120-125.

Papalamprou, E. M., Doxastakis, G. I., Biliaderis, C. G., & Kiosseoglou, V. (2009). Influence on preparation methods on physicochemical and gelation properties of chickpea protein isolates. *Food Hydrocolloids, 23*, 337-343.

Papalamprou, E. M., Doxastakis, G. I., & Kiosseoglou, V. (2010). Chickpea protein isolates obtained by wet extraction as emulsifying agents. *Journal of the Science of Food Agriculture, 90*, 304-313.

Paredes-Lopez, O., Ordorica-Falomir, C., & Olivares-Vazquez, M. R. (1991). Chickpea protein isolates: Physicochemical, functional and nutritional characterization. *Journal of Food Science, 56*, 726-729.

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry, 26*, 716-723.

Pelgrom, P. J. M., Vissers, A. M., Boom, R. M., & Schutyser, M. A. I. (2013). Dry fractionation for production of functional pea protein concentrates. *Food Research International, 53*, 232-239.

Philip, J. C. (1907). Influence of non-electrolytes and electrolytes on the solubility of sparingly soluble gases in water the question of hydrates in solution. *Journal of the Chemical Society, 91*, 711-717.

Polignano, G. B., Uggenti, P., & Scippa, G. (1993). The pattern of genetic diversity in faba bean collections from Ethiopia and Afghanistan. *Genetic Resources and Crop Evolution, 40*, 71-75.

Pozani, S., Doxastakis, G., & Kiosseoglou, V. (2002). Functionality of lupin seed protein isolate in relation to its interfacial behaviour. *Food Hydrocolloids, 16*, 240-247.

Pradhan, A., Shahi, V. K., & Sinha, S. K. (2014). Evaluation of genetic diversity in faba bean (*Vicia faba* L.) genotypes using seed protein and isozymes electrophoresis. *National Academy Science Letters, 37*, 303-309.

Principal component analysis (PCA) and partial least squares (PLS) technical notes. (*StatSoft: Statistica Help*). Retrieved July 29, 2015.

Qayyum, M. M. N., Butt, M. S., Anjum, F. M., & Nawaz, H. (2012). Compositional analysis of some selected legumes for protein isolates recovery. *The Journal of Animal & Plant Sciences, 22*, 1156-1162.

Ragab, D. M., Babiker, E. E., & Eltinay, A. H. (2004). Fractionation, solubility and functional properties of cowpea (*Vigna unguiculata*) proteins as affected by pH and/or salt concentration. *Food Chemistry, 84*, 207-212.

Rangel, A., Domont, G. B., Pedrosa, C., & Ferreira, S. T. (2003). Functional properties of purified vicilins from cowpea (*Vigna unguiculata*) and pea (*Pisum sativum*) and cowpea protein isolate. *Journal of Agriculture and Food Chemistry, 51*, 5792-5797.

Rasband, W. S. (1997-2014). ImageJ. *U. S. National Institutes of Health, Bethesda*, Maryland, USA, http://imagej.nih.gov/ij/.

Reddy, N. R., Pierson, M. D., Sathe, S. K., & Salunkhe, D. K. (1985). Dry bean tannins – a review of nutritional implications. *Journal of the American Oil Chemists' Society, 62*, 54-549.

Robblee, A. R., Clandini, D. R., Hardin, R. T., Milne, G. R., & Darlington, K. (1977). Studies on the use of faba beans in rations for laying hens. *Canadian Journal of Animal Science, 57*, 421-425.

Robertson, L. D., & El-Sherbeeny, M. (1991). Distribution of discreetly scored descriptors in a pure line faba bean (*Vicia faba* L.) germplasm collection. *Euphytica, 57*, 83-92

Roy, F., Boye, J. I., & Simpson, B. K. (2010). Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil. *Food Research International, 43*, 432-442.

Rubiales, D. (2011). The magazine of the European Association for Grain Legumes Research, *Grain Legumes, 56*, 1-36.

Saenz de Miera, L. E., Ramos, J., & Perez de la Vega, M. (2008). A comparative study of convicilin storage protein gene sequences in species of the tribe *Vicieae*. *Genome, 51*, 511-523.

Saharan, K., & Khetarpaul, N. (1994). Protein quality traits of vegetable and field peas: Varietal differences. *Plant Foods for Human Nutrition, 45*, 11-22.

Sathe, S. K., & Salunkhe, D. K. (1981). Functional properties of great northern bean proteins: emulsion, foaming, viscosity & gelation properties. *Journal of Food Science, 46*, 71-84.

Scholz, G., Richter, J., & Manteuffel, R. (1974). Studies on seed globulins from legumes. 1. Separation. *Journal of Plant Physiology, 166*, 163-171.

Schroeder, H. E. (1982). Quantitative studies on the cotyledonary proteins in the genus *Pisum*. *Journal of the Science of Food and Agriculture, 33*, 623-633.

Schutyser, M. A. I., & Van der Goot, A. J. (2011). The potential of dry fractionation processes for sustainable plant protein production. *Trends in Food Science & Technology, 22*, 154-164.

Schutyser, M. A. I., Pelgrom, P. J. M., Van der Goot, A. J., & Boom, R. M. (2015). Dry fractionation for sustainable production of functional legume protein concentrates. *Trends in Food Science and Technology,* 1-11 (in press).

Schwenke, K. D., Knopfe, C., Mikheeva, L. M., & Grinberg, V. Y. (1998). Structural changes of legumin from faba beans (*Vicia faba* L.) by succinylation. *Journal of Agriculture and Food Chemistry, 46*, 2080-2086.

Shahidi, F., & Naczk, M. (2004). Antioxidant properties of food phenolics. In F. Shahidi, & M. Naczk (Ed.), *Phenolics in Food and Nutraceuticals* (pp. 403-437). Boca Raton: CRC Press.

Shen, L., & Tang, C. (2014). Emulsifying properties of vicilins: Dependence on the protein type and concentration. *Food Hydrocolloids, 36*, 278-286.

Sikorski, Z. E. (2001). Functional properties of proteins in food systems. In Z. E. Sikorski (Ed.), *Chemical and Functional Properties of Food Proteins* (pp. 112-135). Boca Raton: CRC Press.

Sillero, J. C., Villegas-Fernandez, A. M., Thomas, J., Rojas-Molina, M. M., Emeran, A. A., Fernandez-Aparicio, M., & Rubiales, D. (2010). Faba bean breeding for disease resistance. *Field Crop Research, 115*, 297-307.

Singh, U. (1988). Antinutritional factors of chickpea and pigeonpea and their removal by processing. *Plant Foods for Human Nutrition, 38*, 251-261.

Singh, K. B., Williams, P. C., & Nakoul, H. (1990). Influence of growing season, location and planting time on some quality parameters of kabuli chickpea. *Journal of the Science of Food and Agriculture, 53*, 429-434.

Soderberg, J. (2013). Functional Properties of legume proteins compared to egg proteins and their potential as egg replacers in vegan food (Master' Thesis, Swedish University of Agricultural Science, Uppsala, Sweden). Retrieved from *http://stud.epsilon.slu.se/6240/.*

Sosulski, F. W., & Youngs, C. G. (1979). Yield and functional properties of air-classified protein and starch fractions from eight legume flours. *Journal of the American Oil Chemists' Society, 56*, 292-295.

Sosulski, F. W., & McCurdy, A. R. (1987). Functionality of flours, protein fractions and isolates from field peas and faba bean. *Journal of Food Science, 52*, 1010-1014.

Sosulski, F. W., Sosulski, K., & McCurdy, A. (1988). Wet milling vs air classification of field peas to isolate protein, starch and fibre. *Acta Alimentaria Polonica, 14*, 41-49.

StatSoft, Inc. (2014). STATISTICA (data analysis software system), version 12. *www.statsoft.com*.

Strzetelski, J. A., Lipianska, E., Bilik, K., Niwinska, B., Osieglowski, S., & Pyska, H. (1996). Effect of bean seeds, lupins and rapeseed as substitutes for protein extracted soybean meal in concentrate mixture on calves' productivity (in Polish). *Roczniki Naukowe Zootechniki, 23*, 131-142.

Sun, T., & Ho, C. (2005). Antioxidant activities of buckwheat extracts. *Food Chemistry, 90*, 743-749.

Sun, X. D., & Arntfield, S. D. (2010). Gelation properties of salt extracted pea protein induced by heat treatment. *Food Research International, 43*, 509-515.

Swanson, B. G. (1990). Pea and lentil protein extract and functionality. *Journal of the American Oil Chemists' Society, 67*, 276-280.

Tcholakova, S., Denkov, N. D., Ivanov, I. B., & Campbell, B. (2006). Coalescence stability of emulsions containing globular milk proteins. *Advances in Colloid and Interface Science, 123*, 259-293.

Tian, S., Kyle, W. S. A., & Small, D. M. (1999). Pilot scale isolation of proteins from field peas (*Pisum sativum*, L.) for use as food ingredients. *International Journal of Food Science and Technology, 34*, 33-39.

Toews, R., & Wang, N. (2013). Physicochemical and functional properties of protein concentrates from pulses. *Food Research International, 52*, 445-451.

Townsend, A., & Nakai, S. (1983). Relationships between hydrophobicity and foaming characteristics of food proteins. *Journal of Food Science, 48*, 588-594.

Tucci, M., Capparelli, R., Costa, A., & Rao, R. (1991). Molecular heterogeneity and genetics of *Vicia faba* seeds storage proteins. *Theoretical and Applied Genetics, 81*, 50-58.

Turro, E. J., & Sipos, E. F. (1970). Soy products in commercial cake formulations. *Baker's Digest, 44*, 58-64.

Tyler, R. T. (1984). Impact milling quality of grain legumes. *Journal of Food Science, 49*, 925-930.

Tyler, R. T., Youngs, C. G., & Sosulski, F. W. (1981). Air classification of legumes. I. Separation efficiency, yield, and composition of the starch and protein fractions. *Cereal Chemistry, 58*, 144-148.

Tzitzikas, E. N., Vincken, J. P., Groot, J., Gruppen, H., & Visser, R. G. (2006). Genetic variation in pea seed globulin composition. *Journal of the Science of Food and Agriculture, 54*, 425-433.

Ustimenko-Bakumovsky, G. V. (1983). *Plant growing in the tropics and subtropics.* New York: Macmillan Publication.

Utsumi, S., Matsumura Y., & Mori, T. (1997). Structure-function relationships of soy proteins. In S. Damodaran, & A. Paraf (Ed.), *Food Proteins and Their Applications* (pp. 257-291). Marcel Dekker: CRC Press.

Van Der Poel, A. F. B. (1990). Effect of processing on anti-nutritional factors and protein nutritional value of dry beans (*Phaseolus vulgaris* L.). A review. *Animal Feed Science and Technology, 29*, 179-208.

Varfolomeeva, E. P., Danilenko, A. N., Bikbov, T. M., Grinberg, V. Ya., & Tolstogusov, V. B. (1985). The rheological properties of diluted solutions of 11S globulin isolated from soybeans by using selective thermal denaturation of 2S and 7S globulins. *Nahrung, 28*, 503-515.

Vernaza, M. G., Dia, V. P., Gonzaalez de Mejia, E., & Chang, Y. K. (2012). Antioxidant and anti-inflammatory properties of germinated and hydrolysed Brazilian soybeans flours. *Food Chemistry, 134*, 2217-2225.

Vidal-Valverde, C., Frias, J., Hernandez, A., Martın-Alvarez, P. J., Sierra, I., & Rodriguez, C. (2003). Assessment of nutritional compounds and antinutritional factors in pea (*Pisum sativum*) seeds. *Journal of Science of Food and Agriculture, 83*, 298-306.

Vidal-Valverde, C., Frias, J., Sierra, I., Blazquez, I., Lambein, F., & Kuo, Y. H. (2002). New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology, 215*, 472-477.

Vilarino, M., Metayer, J. P., Crepon, K., & Duc, G. (2009). Effects of varying vicine, convicine and tannin contents of faba bean seeds (*Vicia faba* L.) on nutritional values for broiler chicken. *Animal Feed Science and Technology, 150*, 114-121.

Vioque, J., Alaiz, M., & Giron-Calle, J. (2012). Nutritional and functional properties of *Vicia faba* protein isolates and related fractions. *Food Chemistry, 132*, 67-72.

Vose, J. R. (1980). Production and functionality of starches and protein isolates from legume seeds (field peas and horse beans). *Cereal Chemistry, 57*, 406-410.

Waggle, D. H., Steinke, F. H., & Shen, J. L. (1989). Isolated soy proteins. In R. H. Matthews (Ed.), *Legumes: Chemistry, Technology and Human Nutrition* (pp. 99-138). New York: Marcel Dekker.

Walstra, P. (2003). *Physical chemistry of foods*. New York: Marcel Dekker.

Wang, H. F., Zong, X. X., Yang, T., Sun, X. L., Ma, Y., & Redden, R. (2012). Genetic diversity and relationship of global faba bean (*Vicia faba* L.) germplasm revealed by ISSR markers. *Theoretical and Applied Genetics, 124*, 789-797.

Wang, P., & Ueberschar, K. H. (1990). The estimation of vicine, convicine and condensed tannins in 22 varieties of faba beans (*Vicia faba* L.). *Animal Feed Science and Technology, 31*, 157-165.

Wang, N., Hatcher, D. W., Warkentin, T., & Toews, R. (2010). Effect of cultivar and environment on physicochemical and cooking characteristics of field pea (*Pisum sativum*). *Food Chemistry*, *118*, 109-115.

Wright, D. J., & Boulter, D. (1974). Purification and subunit structure of legumin of *Vicia faba* L. (Broad bean). *Biochemical Journal, 141*, 413-418.

Zheng, B-A., Matsumura, Y., & Mori, T. (1992). Molecular forces in thermal association-dissociation and gelation of legumin from broad beans. *Journal of Food Science, 57, 423*-426.

Zheng, G. H., Sosulski, F. W., & Tyler, R. T. (1998). Wet-milling, composition and functional properties of starch and protein isolated from buckwheat groats. *Food Research International, 30*, 493-50.

Zong, X., Liu, X., Guan, J., Wang, S., Liu, Q., Paull, J. G., & Redden, R. (2009). Molecular variation among Chinese and global winter faba bean germplasm. *Theoretical and Applied Genetics, 118*, 971-978.

Zong, X., Ren, J., Guan, J., Wang, S., Liu, Q., Paull, J. G., & Redden, R. (2010). Molecular variation among Chinese and global germplasm in spring faba bean areas. *Plant Breeding*, *129*, 508-513.