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Effect of Processing on Mungbean (*Vigna radiata*) Flour Nutritional Properties and Protein Composition

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Abstract

Pulses are traditionally processed prior to consumption, providing opportunities for modifying nutritional composition, dependant on the type of pulse and method used. In this study, we investigated the effect of whole seed, dehulling (dahl), germination and roasting on changes in mungbean flour nutritional properties, protein composition and relative protein abundance. Processed flours were analysed and compared for protein content, moisture, fat, ash, dietary fibre, total starch and amylose. Significant differences were imparted on dietary fibre content, with roasting and germination increasing the ratio of insoluble/soluble fibre as well as resistant starch. Comparative proteomic analysis resulted in a combined total of 539 protein identifications, searching against the Mungbean reference genome (NCBI *Vigna radiata* Annotation Release 100). Normalised spectral abundance factors were used as a measure of relative abundance and statistical analysis was applied (Students' T-Test), where proteins with a p-value of < 0.05 considered significantly different. Processing imparted considerable changes to nutritional composition and should be further exploited for food applications. The comparative proteomic analyses carried out in this study proved useful for investigating the effect of processing on subsequent changes in protein composition and relative abundance.

Keywords: mungbean, processing, flour, nutrition, protein, proteomics

1. Introduction

Pulses can be used for enhancing the nutritional and functional properties of food, providing a source of protein, carbohydrate, dietary fibre, vitamins and minerals (Duranti & Gius, 1997; Prakash et al., 2001; Tharanathan & Mahadevamma, 2003; Boye et al., 2010; Nair et al., 2013; Vaz Patto et al., 2015). Major types of pulses grown in Australia include chickpea, faba bean, field pea, lentil, lupin and mungbean. The transformation of pulses from a commodity crop, to healthy value-added food ingredients, would benefit grain producers, processors and consumers. Promoting the health benefits of pulses, combined with improved nutritional qualities, functionality and diversity of food applications may lead to greater consumer acceptance, consumption and sustainable food production.

Mungbean (*Vigna radiata*) is primarily grown in rotation with cereals, delivering agronomic benefits, such as fixing atmospheric nitrogen into the soil. The majority of mungbean grown in Australia is exported (around 95%), consisting predominantly of the large seeded Crystal variety, preferable for cooking and processing markets. Seed quality characteristics for export markets include size, colour, uniformity, varietal purity and protein content, which can vary depending on varietal performance, environment, agronomic practices and processing conditions. A review of the literature reported wide variation in protein content, averaging 23.8 g 100 g^{-1} (Dahiya et al., 2015). Recently, a survey of Australian mungbean varieties grown in different regions reported variation in protein content, ranging from 23.6 to 30.1 g 100 g^{-1}, with the proportion of essential amino acids found to be highly conserved, comprising 38.1 to 38.7% of total protein (Skylas et al., 2017).

Processing of pulses prior to consumption provides an opportunity for modifying nutritional properties, reducing the level of anti-nutritional factors, increasing protein digestibility and bioavailability of nutrients, as well as improving functionality, flavour and aroma (Tharanathan & Mahadevamma, 2003; Vaz Patto et al., 2015; Patterson et al., 2017). Primary processing of pulses includes the more conventional methods such as soaking, dehulling, splitting and milling to flour for a range of applications. Secondary processing methods include a range of diverse treatments and include roasting, toasting, germination, fermentation and extrusion. Pulse flours can be further fractionated using wet or dry processes, for production of concentrated protein flours and isolates, which can also be modified to produce a range of functional ingredients (Fan & Sosulski, 1974; Thompson, 1977; Rahma et al., 2000; Li et al., 2010; Wang et al., 2011; Pelgrom et al., 2015).

Proteomic technologies provide a range of methods for characterising changes in protein composition, relative abundance and protein identification (Thelen & Peck, 2007; Matros et al., 2011). Aspects of this technology have previously been applied to mungbean for characterising changes in protein expression during seed development, germination and protein isolation (Ghosh & Pal, 2012; Kazlowski et al., 2013; Skylas et al., 2017). Advances in comparative proteomics include 'label-free' quantitation known as spectral counting (SC), based on counting the number of spectra identified for peptides of specific proteins, used as a proxy for protein abundance (Lundgren et al., 2010; Neilson et al., 2013). Resulting spectra is then used to interrogate protein sequence databases to infer identification. Abundantly expressed proteins, such as seed storage proteins, will produce more spectra, resulting in more peptides belonging to that particular protein being identified, which is then used as a measure relative abundance (Liu et al., 2004; Zhang et al., 2006; Zybailov et al., 2007; Neilson et al., 2013). Improvements in the quality of SC data were made with the application of normalised spectral abundance factors (NSAFs), which are applied to account for the length of individual proteins, enabling comparison and statistical analysis of relative protein abundance (Zybailov et al., 2006; Zybailov et al., 2007; Mosley et al., 2009; Podwojski et al., 2010; Neilson et al., 2013; Mirzaei et al., 2016).

The objective of this study was to determine the effect of selected primary and secondary processes such as dehulling, germination and roasting on the nutritional composition of mungbean flours, as well as subsequent changes in protein composition and relative abundance. This study provides further knowledge of mungbean protein composition and subsequent changes associated from processing.

2. Methods

2.1 Seed Material and Milling

Mungbean whole seed, raw dahl and roasted dahl were commercially processed and provided by the Blue Ribbon Group (Richlands, QLD 4077), produced from the large seeded Crystal variety. For germination of whole seed material, seed was cleaned in absolute ethanol for 1 min, then rinsed three times with water and drained. Fresh water was then added and seed material was allowed to soak and imbibe for 12 hours. Seeds were rinsed again, drained and germinated for 48 hours in an incubator (at 22 °C). Germinated seeds (including hulls) were oven dried (50 °C) and thrashed over a 2 mm sieve screen to dislodge seedling shoots, which were then separated and discarded. Seed material was milled to flour using an Alpine Pin Mill and designated herein as mungbean whole seed flour (MWF), raw dahl flour (MDF), roasted dahl flour (MRF) and germinated flour (MGF).

2.2 Nutritional Composition

Nutritional testing was carried out at the NATA accredited AEGIC Analytical Laboratory (Sydney) using approved standard methods of analysis. Testing was carried out in duplicate and the averaged result was reported. Nitrogen content was determined by the Dumas method using a LECO TruMac protein analyser (AOAC 992.23) and converted to protein (N × 6.25). Standard methods used included ash; AOAC 923.03 and AACC 08-01.01, moisture; AOAC 925.10 and AACC 44-15.02, fat; AACC 30-10.01, and total dietary fibre (TDF; insoluble and soluble); AOAC 985.29 and 991.42. Starch was measured by Megazyme Starch Assay Kit (AOAC 996.11 and AACC 76-13.01). Resistant starch was measured by Megazyme Resistant Starch Assay Kit (AOAC 2002.02 and AACC 32-40.01).

2.3 Comparative Proteomic Analysis

The comparative proteomic methodology used in this study was the same as previously reported by the authors (Skylas et al., 2017). Flour samples were solubilised in 50 mM TEAB containing 0.5% SDS and probe sonicated, reduced (using dithiothreitol) and alkylated (iodoacetamide). Samples were digested with trypsin for 16 hours at 37°C and SDS was removed from the digested samples using a detergent removal kit followed by a C18 clean up. Samples were dried down, resuspended in 0.1% formic acid and used for analysis. Analysis was carried out by

reversed phase nano-LC directly coupled in line with a MS/MS system (LC-MS/MS). Samples from each fraction were separated over 90 minute gradients using an Easy Nano LC 1000 (Thermo Scientific). Samples (10 μL) were injected onto an 'in house' packed solid core Halo C18 100 μm × 3 cm peptide trap column and desalted with 20 μL of 0.1% formic acid. The peptide trap was switched on line with the C18 75 μm × 10 cm analytical reversed phase column. Peptides were eluted from the column using a linear solvent gradient, step-wise from 5-25% of buffer [99.9% (v/v) acetonitrile, 0.1% (v/v) formic acid] for 80 min, 25-85% of buffer for 2 min and then held at 85% for 8 min at a flow rate of 300 L/min across the gradient.

The column eluate was directed into a nanospray ionization source of the QExactive mass spectrometer (ThermoScientific) and a 1.5 kV electrospray voltage was applied via a liquid junction upstream of the column. Resulting spectra were scanned over the range 350-2000 amu. Automated peak recognition, dynamic exclusion, and MS/MS of the top ten most intense precursor ions at 30% normalised collision energy were performed. The LC-MS/MS spectra were searched using the MS software Mascot (Matrix Science, London, UK), against the Mungbean reference genome (NCBI Vigna radiata Annotation Release 100) containing 35143 entries (Kang et al., 2014). Peptides were identified with a 1% false-discovery rate from a concatenated forward-reversed database search. Significant peptide matches were exported and samples compared using NSAF with the program referred to as "SCRappy" (Neilson et al., 2013). Proteins with p-values < 0.05 following Student's T-Test of NSAF were considered significantly different between groups.

3. Results and Discussion

3.1 Nutritional Composition of Processed Mungbean Flours

Nutritional composition of respective mungbean flours (MWF, MDF, MRF and MGF) were analysed in duplicate and the average result for each nutritional component is reported in Table 1. The process of dehulling removes the outer seed coat from the cotyledon, which reportedly comprises ~12% of dry seed weight (Singh et al., 1968), producing dahl with improved palatability and cooking time, used in a range of food applications. The effect of dehulling on nutritional composition was determined by comparison of MWF and MDF, primarily resulting in decreased dietary fibre content, from 10.6 to 4.6 g 100 g^{-1}, respectively. Dehulling also reduced ash content, by removal of mineral content present in the outer seed coat. The effect of germination on nutritional composition was determined by comparison of MWF and MGF, with germination increasing protein and dietary fibre content, altering the ratio of insoluble (IDF) to soluble dietary fibre (SDF). Changes in the dietary fibre content most likely resulting from enzymatic modification of cell wall polysaccharides during germination, consistent with previous reports of increased crude fibre in lupins and dietary fibre in peas (Martín-Cabrejas et al., 2003; James et al., 2012). However, changes in nutritional composition can be partly attributed to decreased starch content, resulting from enzyme hydrolysis during germination, required to provide a source of energy for the emerging seedling. The proportion of amylose in starch also decreased in MGF, with similar findings observed for germinated lentil and horsegram flours (Ghumman et al., 2016). Roasting is often used for enhancing nutritional qualities, flavour and aroma, with the effect on nutritional composition determined by comparison of MDF and MRF. Roasting imparted significant changes in the ratio of IDF/SDF, increasing from 2.5 to 16, for MDF and MRF, respectively. The increased resistant starch content of MRF is most likely due to high-temperature induced modification of starch structure, increasing resistance to starch-degrading enzymes (Li et al., 2011).

Table 1. Nutritional composition of processed mungbean flours (g 100 g^{-1}). Protein and starch were corrected for moisture content and reported on a dry basis

Composition	MWF	MDF	MRF	MGF
Protein	27.6	28.3	27.8	29.4
Moisture	9.9	11.3	5.9	6.0
Fat	1.9	1.8	2.0	2.0
Ash	3.5	3.1	3.1	3.2
Dietary fibre:				
TDF	10.6	4.6	3.4	13.1
IDF	7.6	3.3	3.2	11.6
SDF	3.0	1.3	0.2	1.5
IDF/SDF ratio	2.5	2.5	16	7.7
Resistant starch	3.0	0.7	14.6	4.0
Starch	45.4	53.1	51.9	42.6
Amylose (%)	37.6	39.4	38.4	33.1

3.2 Comparative Proteomic Analysis of Processed Mungbean Flours

A comparative proteomic approach was applied to the representative processed flour samples, in order to investigate the effect of dehulling, germination and roasting on changes in protein composition and relative protein abundance. A combined total of 539 proteins were identified in the processed flours, with the distribution of these proteins between flours represented by a four-way Venn diagram (Figure 1). From this total, 72 proteins were classified as common, being present in MWF, MDF, MRF and MGF. Inferred protein identifications were reported in terms of matching protein description, identifiable database accession, average SC and NSAFs, as well as the ratio of NSAFs between two flour samples, used as measure of relative abundance. Statistical analyses (Student's T-Test) of NSAFs was applied, and proteins with a p-value < 0.05 considered significantly different in abundance. The dataset was filtered, by removal of inferred protein identifications containing < 5 SC, except for those comparisons in which one sample contained >5 SC. The absence of a specific protein in any sample does not conclusively mean that protein is not present, but only that the abundance level is too low to be detected on 5 or more occasions (as specified by our reporting criteria).

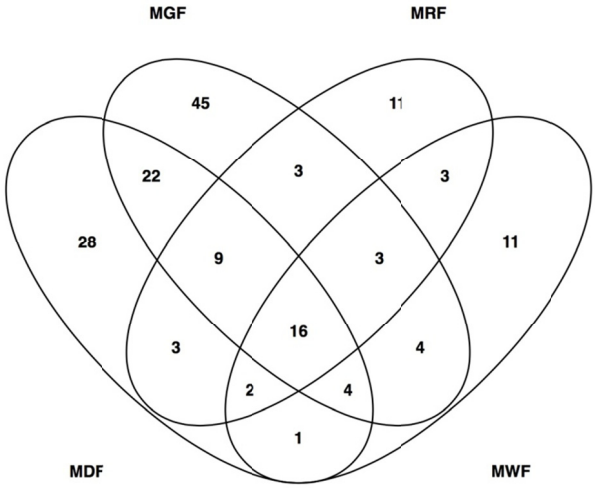

Figure 1. Four-way Venn diagram representing the distribution of identified proteins between processed mungbean flours (MWF, MDF, MRF and MGF)

3.3 Effect of Dehulling on Protein Composition and Relative Abundance

The effect of dehulling on protein composition and relative abundance was determined by comparison of proteins identified for MWF (95 proteins) and MDF (164 proteins). Of these proteins, 88 were classified as common, in which 27 were found to be significantly different in relative abundance, reported in Table 2, sorted in descending order of NSAFs. Identified proteins classified as specific for either MWF or MDF are listed in Appendix A. The most abundant proteins identified were the globulin storage proteins, which comprise mostly of the 8S, and to a lesser extent 11S and 7S globulins, reportedly having high sequence homology to soybean conglycinin and storage proteins from other pulses (Mendoza et al., 2001; Bernardo et al., 2004; Liu et al., 2015). Storage proteins, reported in Table 2, were predominantly identified as beta-conglycinin beta chain-like, with some found to be significantly different in relative abundance between the two flours. Changes in abundance of storage proteins could be attributed to the increased protein content of MDF compared to MWF (Table 1).

Another group of proteins found to be abundant were the late embryogenesis abundant (LEA) proteins, which accumulate in the seed embryo, playing a protective role through protein-protein interactions, in response to water deficit related stress and seed dehydration (Battaglia & Covarrubias, 2013). There were six matches to this group of proteins, consisting of three different types (LEA type D-29, D-34 and 2), with the majority of these proteins being more abundant in MWF, two of which were significantly different. This may result from the loss of seed embryo tissue during the dehulling and kibbling process, resulting in the reduction of embryonic proteins in MDF. This is further supported by two other embryonic proteins (DC-8-like), which were found to be less abundant in MDF.

3.4 Effect of Germination on Protein Composition and Relative Abundance

During the germination process, increased enzyme activity leads to modification of nutritional composition, resulting from hydrolysis of macronutrients such as starch and protein (Nout & Ngoddy, 1997). This process can be used to enhance nutritional composition, resulting in increased protein solubility and digestibility (James et al., 2012). The effect of germination on protein composition and relative abundance was determined by comparison of proteins identified for MWF (95 proteins) and MGF (169 proteins). Of these proteins, 85 were classified as common, in which, 27 were found to be significantly different in relative abundance, reported in Table 3, sorted in descending order of NSAFs. Identified proteins classified as specific for either MWF or MGF are listed in Appendix B.

Of those proteins that were significantly different between MGF and MDF, there were 6 enzymes, involved in metabolism, which were more abundant in MGF. These included enzymes involved in starch degradation and carbon metabolism, including alpha-1,4 glucan phophorylase, UTP-glucose-1-phosphate uridylyltransferase, fructose-bisphosphate aldolase and ATPase (subunit 1). The other two enzymes included seed linoleate 9S-lipoxygenase, involved in fatty acid metabolism (Aanangi et al., 2016) and nudix hydrolase 3-like, which is involved in hydrolysis of a wide range of organic pyrophosphates and has been implicated to play a role in germination of Arabidopsis (Zeng et al., 2014). Other enzymes involved in metabolism, detected only in MGF (Appendix B), were glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase, fructokinase-2-like and enolase.

Heat shock proteins (HSP) were also found to be significantly different between MGF and MDF. These proteins act as molecular chaperones, assisting in cellular processes including folding, assembly and degradation of proteins, as well as stabilisation and refolding of proteins in response to stress related conditions (Wang et al., 2004). HSPs found to be more abundant in MGF included HSP 70 kDa, HSP cognate 70 kDa protein 2 and HSP 83. The HSP cognate 70 kDa protein had the second highest ratio of NSAFs between MGF/MDF (at 7.28714), with the luminal-binding protein, also thought to function as a chaperone, having the highest ratio of NSAFs (at 7.64959). This is indicative of these proteins playing a crucial role in protein synthesis and degradation during the early stages of germination. Small HSPs (17.6 kDa) have previously been identified in germinated mungbean cotyledons, also thought to play a protective role during this process (Ghosh & Pal, 2012).

3.5 Effect of Roasting on Protein Composition and Relative Abundance

High temperatures required for roasting can partially denature, aggregate and modify protein structures, including glycation of lysine and free amines, resulting from Maillard reactions (Walker & Kochhar, 1982; Sun-Waterhouse et al., 2014; Wang et al., 2016). Such protein modifications may reduce the potential number of tryptic peptides generated during the protein digestion step, carried out prior to LC-MS/MS, potentially leading to an under-estimation of the relative abundance of these proteins, compared to raw or non-roasted samples. The effect of roasting on protein composition and relative abundance was determined by comparison of proteins identified for MDF (164 proteins) and MRF (111 proteins). Of these proteins, 105 were classified as common, in which, 14 were found to be significantly different in relative abundance, reported in Table 4, sorted in descending order of NSAFs. Identified proteins classified as specific for either MWF or MRF are listed in Appendix C.

Table 2. Inferred identity and relative abundance of those proteins classified as common for MDF and MWF. NSAF ratios of MDF/MWF that are statistically significant ($p < 0.05$) are highlighted in bold

Identifier	SC		Matching protein description	NSAF		Ratio
	MDF	MWF	[*Vigna radiata* var. *radiata*]	MDF	MWF	MDF/MWF
gi|951066354|ref|XP_014523937.1|	557	250	Beta-conglycinin, beta chain-like isoform X1	0.09288	0.11023	**0.84260**
gi|951067727|ref|XP_014524354.1|	546	236	Beta-conglycinin, beta chain-like	0.09161	0.10434	0.87801
gi|951002540|ref|XP_014507363.1|	334	147	Beta-conglycinin, beta chain-like	0.05910	0.06893	**0.85740**
gi|950940165|ref|XP_014492536.1|	307	91	Beta-conglycinin, beta chain-like	0.05389	0.04237	**1.27177**
gi|951033982|ref|XP_014515878.1|	252	84	Beta-conglycinin, beta chain-like	0.04225	0.03746	1.12777
gi|951056419|ref|XP_014521758.1|	336	108	Glycinin G4-like	0.04171	0.03576	1.16650
gi|951066351|ref|XP_014523936.1|	261	56	Beta-conglycinin, alpha~ chain-like	0.03694	0.02102	**1.75759**
gi|951023258|ref|XP_014513134.1|	83	29	Albumin-2-like	0.02841	0.02696	1.05354
gi|951066358|ref|XP_014523938.1|	152	130	Beta-conglycinin, beta chain-like isoform X2	0.02525	0.05784	0.43648
gi|950930231|ref|XP_014503883.1|	71	35	Dehydrin DHN3-like	0.02326	0.03062	**0.75981**
gi|950951134|ref|XP_014495577.1|	66	33	Embryonic protein DC-8-like	0.01723	0.02303	**0.74844**
gi|950951230|ref|XP_014495608.1|	22	15	18 kDa seed maturation protein-like	0.01711	0.03232	**0.52929**

Identifier	SC		Matching protein description	NSAF		Ratio
	MDF	MWF	[*Vigna radiata* var. *radiata*]	MDF	MWF	MDF/MWF
gi|951066718|ref|XP_014524029.1|	42	28	P24 oleosin isoform B	0.01634	0.02827	**0.57786**
gi|951034870|ref|XP_014516158.1|	19	16	Protein SLE2	0.01555	0.03407	**0.45645**
gi|951005658|ref|XP_014508213.1|	61	38	Low quality protein: late embryogenesis abundant protein D-29	0.01231	0.02021	**0.60907**
gi|951042174|ref|XP_014518107.1|	66	24	Basic 7S globulin 2-like	0.01230	0.01194	1.02980
gi|951067725|ref|XP_014524353.1|	39	10	Beta-conglycinin, beta chain-like, partial	0.01218	0.00892	**1.36493**
gi|950959908|ref|XP_014497548.1|	15	6	Protein SLE1 isoform X1	0.01106	0.01151	0.96135
gi|951016290|ref|XP_014511078.1|	67	27	Embryonic protein DC-8-like	0.01066	0.01138	0.93635
gi|951072910|ref|XP_014491941.1|	14	6	Non-specific lipid-transfer protein 1	0.00995	0.01262	0.78870
gi|951032441|ref|XP_014515393.1|	18	8	Desiccation protectant protein Lea14 homolog	0.00964	0.01110	0.86838
gi|951006474|ref|XP_014508481.1|	23	8	1-Cys peroxiredoxin	0.00817	0.00764	1.06875
gi|950968931|ref|XP_014499690.1|	86	12	Seed linoleate 9S-lipoxygenase-3	0.00768	0.00281	2.72783
gi|951066347|ref|XP_014523935.1|	22	19	Late embryogenesis abundant protein D-34-like	0.00754	0.01741	**0.43320**
gi|950974150|ref|XP_014500866.1|	8	4	Uncharacterised protein LOC106761813	0.00750	0.01079	0.69518
gi|950973966|ref|XP_014500828.1|	6	5	Uncharacterised protein LOC106761773	0.00741	0.01675	**0.44233**
gi|951021491|ref|XP_014512682.1|	44	19	Sucrose-binding protein-like	0.00661	0.00772	0.85599
gi|951056290|ref|XP_014521723.1|	21	12	Uncharacterised protein LOC106778296	0.00658	0.01027	**0.64056**
gi|951000293|ref|XP_014506761.1|	23	5	Glucose and ribitol dehydrogenase homolog 1-like	0.00651	0.00399	**1.63155**
gi|950985610|ref|XP_014503380.1|	7	1	Uncharacterised protein LOC106763730	0.00593	0.00298	1.99007
gi|951035730|ref|XP_014516424.1|	10	2	Nucleoside diphosphate kinase 1	0.00551	0.00391	1.40732
gi|950986379|ref|XP_014503555.1|	10	5	17.5 kDa class I heat shock protein-like	0.00512	0.00753	**0.68041**
gi|951023922|ref|XP_014513287.1|	18	4	Glucose and ribitol dehydrogenase homolog 1-like	0.00511	0.00348	1.47009
gi|951040313|ref|XP_014517640.1|	17	6	Glucose and ribitol dehydrogenase homolog 1-like	0.00481	0.00436	1.10382
gi|950969621|ref|XP_014499874.1|	6	1	Late embryogenesis abundant protein 2	0.00472	0.00221	2.13426
gi|951014217|ref|XP_014510496.1|	36	8	Heat shock 70 kDa protein	0.00448	0.00282	1.58885
gi|950950919|ref|XP_014495514.1|	8	3	18 kDa seed maturation protein-like	0.00417	0.00478	0.87327
gi|950993033|ref|XP_014504815.1|	20	7	Alcohol dehydrogenase 1-like [Vigna radiata var. radiata]	0.00416	0.00388	1.07190
gi|951048093|ref|XP_014519608.1|	35	14	Canavalin	0.00398	0.00425	0.93506
gi|951022780|ref|XP_014513011.1|	12	4	Peroxygenase	0.00389	0.00410	0.95109
gi|950943234|ref|XP_014493768.1|	8	7	Peptidyl-prolyl cis-trans isomerase 1	0.00383	0.00933	**0.41080**
gi|951023386|ref|XP_014513168.1|	10	5	Late embryogenesis abundant protein D-34-like isoform X1	0.00327	0.00502	0.65205
gi|951039676|ref|XP_014517471.1|	9	4	Late embryogenesis abundant protein 2-like	0.00324	0.00386	0.84021
gi|950968907|ref|XP_014499686.1|	36	9	Seed linoleate 9S-lipoxygenase-2	0.00322	0.00212	1.51668
gi|951023264|ref|XP_014513135.1|	10	1	Uncharacterised protein LOC106771650	0.00317	0.00096	3.28547
gi|951040842|ref|XP_014517782.1|	5	3	40S ribosomal protein S14	0.00308	0.00441	0.69921
gi|951023134|ref|XP_014513100.1|	6	1	UPF0098 protein TC_0109-like	0.00306	0.00184	1.66134
gi|951065326|ref|XP_014523717.1|	18	1	Luminal-binding protein, partial	0.00304	0.00052	**5.81445**
gi|951066326|ref|XP_014523928.1|	23	16	Beta-conglycinin, beta chain-like	0.00294	0.00538	**0.54616**
gi|950925699|ref|XP_014494982.1|	14	3	Formate dehydrogenase 1, mitochondrial-like isoform X1	0.00279	0.00200	1.39739
gi|950973879|ref|XP_014500811.1|	6	4	Late embryogenesis abundant protein D-34-like	0.00274	0.00467	**0.58811**
gi|950994560|ref|XP_014505167.1|	13	2	UTP--glucose-1-phosphate uridylyltransferase	0.00233	0.00095	2.44624
gi|950974705|ref|XP_014500967.1|	22	2	Nudix hydrolase 3-like	0.00233	0.00077	**3.01850**
gi|950933283|ref|XP_014511756.1|	28	6	Alpha-1,4 glucan phosphorylase L isozyme	0.00223	0.00126	**1.76041**
gi|950951948|ref|XP_014495815.1|	17	1	Heat shock cognate 70 kDa protein 2	0.00216	0.00050	4.33791
gi|950993253|ref|XP_014504861.1|	9	1	Fructose-bisphosphate aldolase, cytoplasmic isozyme	0.00205	0.00109	1.88943
gi|950954866|ref|XP_014496519.1|	5	3	40S ribosomal protein S8-like	0.00193	0.00350	0.55184
gi|951021073|ref|XP_014512575.1|	13	2	Granule-bound starch synthase 1, chloroplastic/amyloplastic-like	0.00169	0.00073	**2.31510**
gi|323149044|ref|YP_004222824.1|	10	2	ATPase subunit 1 (mitochondrion)	0.00164	0.00102	1.60306
gi|950933029|ref|XP_014511428.1|	7	2	Actin-1-like	0.00155	0.00153	1.01112
gi|950934982|ref|XP_014515635.1|	13	2	Heat shock protein 83	0.00149	0.00062	**2.39935**
gi|951072874|ref|XP_014491920.1|	7	3	60S ribosomal protein L4	0.00145	0.00160	0.90941
gi|951067792|ref|XP_014524384.1|	6	5	Seed biotin-containing protein SBP65-like	0.00141	0.00333	**0.42366**
gi|950979939|ref|XP_014501963.1|	8	3	Protein disulfide-isomerase-like	0.00119	0.00143	0.83318
gi|951028515|ref|XP_014514444.1|	6	4	Serine carboxypeptidase-like	0.00100	0.00168	0.59728
gi|951027555|ref|XP_014514203.1|	6	3	ATP synthase subunit beta, mitochondrial	0.00099	0.00135	0.73383
gi|950945335|ref|XP_014494232.1|	9	6	Poly [ADP-ribose] polymerase 3	0.00086	0.00162	**0.53315**
gi|950929466|ref|XP_014502187.1|	11	8	TSC22 domain family protein 1-like	0.00081	0.00158	**0.51206**
gi|950975966|ref|XP_014501194.1|	5	1	Elongation factor 2	0.00053	0.00036	1.47814
gi|950963716|ref|XP_014498402.1|	5	1	Cell division cycle protein 48 homolog	0.00049	0.00038	1.27505

Table 3. Inferred identity and relative abundance of those proteins classified as common for MGF and MWF. NSAF ratios of MGF/MWF that are statistically significant ($p < 0.05$) are highlighted in bold

Identifier	SC		Matching protein description	NSAF		Ratio
	MGF	MWF	[*Vigna radiata* var. *radiata*]	MGF	MWF	MGF/MWF
gi|951066354|ref|XP_014523937.1|	487	250	Beta-conglycinin, beta chain-like isoform X1	0.10618	0.11092	0.95726
gi|951067727|ref|XP_014524354.1|	470	236	Beta-conglycinin, beta chain-like	0.10337	0.10500	0.98451
gi|951002540|ref|XP_014507363.1|	306	147	Beta-conglycinin, beta chain-like	0.07087	0.06936	1.02176
gi|950940165|ref|XP_014492536.1|	303	91	Beta-conglycinin, beta chain-like	0.06965	0.04264	**1.63339**
gi|951066358|ref|XP_014523938.1|	280	130	Beta-conglycinin, beta chain-like isoform X2	0.05960	0.05811	1.02567
gi|951066351|ref|XP_014523936.1|	218	56	Beta-conglycinin, alpha~ chain-like	0.04027	0.02115	**1.90342**
gi|951033982|ref|XP_014515878.1|	169	84	Beta-conglycinin, beta chain-like	0.03715	0.03770	0.98545
gi|951056419|ref|XP_014521758.1|	209	108	Glycinin G4-like	0.03387	0.03598	0.94140
gi|951023258|ref|XP_014513134.1|	69	29	Albumin-2-like	0.03112	0.02714	1.14675
gi|951072910|ref|XP_014491941.1|	16	6	Non-specific lipid-transfer protein 1	0.01517	0.01270	1.19439
gi|951067725|ref|XP_014524353.1|	32	10	Beta-conglycinin, beta chain-like, partial	0.01355	0.00898	1.50821
gi|951005658|ref|XP_014508213.1|	46	38	Low quality protein: late embryogenesis abundant protein D-29	0.01201	0.02034	**0.59024**
gi|951042174|ref|XP_014518107.1|	48	24	Basic 7S globulin 2-like	0.01169	0.01202	0.97246
gi|951006474|ref|XP_014508481.1|	23	8	1-Cys peroxiredoxin	0.01099	0.00769	1.42848
gi|950951134|ref|XP_014495577.1|	24	33	Embryonic protein DC-8-like	0.00846	0.02317	**0.36491**
gi|950968931|ref|XP_014499690.1|	74	12	Seed linoleate 9S-lipoxygenase-3	0.00843	0.00283	2.97410
gi|951021491|ref|XP_014512682.1|	39	19	Sucrose-binding protein-like	0.00777	0.00777	0.99942
gi|951056290|ref|XP_014521723.1|	18	12	Uncharacterised protein LOC106778296	0.00765	0.01033	0.73985
gi|950930231|ref|XP_014503883.1|	17	35	Dehydrin DHN3-like	0.00731	0.03081	**0.23738**
gi|951069682|ref|XP_014490344.1|	7	1	Non-specific lipid-transfer protein 1-like	0.00710	0.00340	**2.08549**
gi|951066718|ref|XP_014524029.1|	13	28	P24 oleosin isoform B	0.00687	0.02846	**0.24130**
gi|951016290|ref|XP_014511078.1|	33	27	Embryonic protein DC-8-like	0.00666	0.01146	0.58153
gi|950943234|ref|XP_014493768.1|	10	7	Peptidyl-prolyl cis-trans isomerase 1	0.00649	0.00939	**0.69122**
gi|951000293|ref|XP_014506761.1|	18	5	Glucose and ribitol dehydrogenase homolog 1-like	0.00648	0.00401	1.61332
gi|950985610|ref|XP_014503380.1|	6	1	Uncharacterised protein LOC106763730	0.00638	0.00300	2.12371
gi|950986379|ref|XP_014503555.1|	10	5	17.5 kDa class I heat shock protein-like	0.00637	0.00758	0.84019
gi|951023922|ref|XP_014513287.1|	17	4	Glucose and ribitol dehydrogenase homolog 1-like	0.00635	0.00350	1.81425
gi|951035730|ref|XP_014516424.1|	8	2	Nucleoside diphosphate kinase 1	0.00544	0.00394	1.38196
gi|951014217|ref|XP_014510496.1|	31	8	Heat shock 70 kDa protein	0.00496	0.00284	**1.75058**
gi|950951230|ref|XP_014495608.1|	4	15	18 kDa seed maturation protein-like	0.00475	0.03253	**0.14606**
gi|951032441|ref|XP_014515393.1|	7	8	Desiccation protectant protein Lea14 homolog	0.00475	0.01117	**0.42508**
gi|951040313|ref|XP_014517640.1|	13	6	Glucose and ribitol dehydrogenase homolog 1-like	0.00454	0.00439	1.03534
gi|951034870|ref|XP_014516158.1|	4	16	Protein SLE2	0.00452	0.03429	**0.13190**
gi|950993253|ref|XP_014504861.1|	14	1	Fructose-bisphosphate aldolase, cytoplasmic isozyme	0.00428	0.00109	**3.91765**
gi|951040842|ref|XP_014517782.1|	5	3	40S ribosomal protein S14	0.00404	0.00444	0.90995
gi|951065326|ref|XP_014523717.1|	18	1	Luminal-binding protein, partial	0.00401	0.00052	**7.64959**
gi|950968907|ref|XP_014499686.1|	33	9	Seed linoleate 9S-lipoxygenase-2	0.00385	0.00214	**1.80187**
gi|950993033|ref|XP_014504815.1|	14	7	Alcohol dehydrogenase 1-like	0.00375	0.00391	0.96008
gi|951022780|ref|XP_014513011.1|	8	4	Peroxygenase	0.00366	0.00412	0.88662
gi|950951948|ref|XP_014495815.1|	23	1	Heat shock cognate 70 kDa protein 2	0.00364	0.00050	**7.28714**
gi|950954866|ref|XP_014496519.1|	7	3	40S ribosomal protein S8-like	0.00354	0.00353	1.00317
gi|951048093|ref|XP_014519608.1|	23	14	Canavalin	0.00338	0.00428	0.78992
gi|951066326|ref|XP_014523928.1|	19	16	Beta-conglycinin, beta chain-like	0.00316	0.00541	**0.58280**
gi|950974705|ref|XP_014500967.1|	21	2	Nudix hydrolase 3-like	0.00290	0.00078	**3.73366**
gi|950977676|ref|XP_014501534.1|	6	3	14-3-3-like protein isoform X1	0.00269	0.00303	0.88821
gi|323149044|ref|YP_004222824.1|	13	2	ATPase subunit 1 (mitochondrion)	0.00268	0.00103	**2.60508**
gi|950925699|ref|XP_014494982.1|	9	3	Formate dehydrogenase 1, mitochondrial-like isoform X1	0.00259	0.00201	1.28961
gi|951066347|ref|XP_014523935.1|	5	19	Late embryogenesis abundant protein D-34-like	0.00248	0.01751	**0.14150**
gi|950933283|ref|XP_014511756.1|	22	6	Alpha-1,4 glucan phosphorylase L isozyme	0.00227	0.00127	**1.78654**
gi|951072874|ref|XP_014491920.1|	8	3	60S ribosomal protein L4	0.00215	0.00161	1.33832
gi|950934982|ref|XP_014515635.1|	15	2	Heat shock protein 83	0.00211	0.00062	**3.39005**
gi|950994560|ref|XP_014505167.1|	9	2	UTP--glucose-1-phosphate uridylyltransferase	0.00196	0.00096	**2.04212**
gi|951027555|ref|XP_014514203.1|	9	3	ATP synthase subunit beta, mitochondrial	0.00185	0.00136	1.35875
gi|950933029|ref|XP_014511428.1|	5	2	Actin-1-like	0.00149	0.00154	0.97032
gi|950979939|ref|XP_014501963.1|	7	3	Protein disulfide-isomerase-like	0.00135	0.00144	0.93825
gi|951028515|ref|XP_014514444.1|	5	4	Serine carboxypeptidase-like	0.00104	0.00169	0.61560
gi|951021073|ref|XP_014512575.1|	5	2	Granule-bound starch synthase 1, chloroplastic/amyloplastic-like	0.00096	0.00073	1.30544

Identifier	SC		Matching protein description	NSAF		Ratio
	MGF	MWF	[*Vigna radiata* var. *radiata*]	MGF	MWF	MGF/MWF
gi|950952971|ref|XP_014496061.1|	5	1	Chaperone protein ClpB1	0.00065	0.00034	1.87751
gi|951023386|ref|XP_014513168.1|	1	5	Late embryogenesis abundant protein D-34-like isoform X1	0.00063	0.00505	**0.12566**
gi|950945335|ref|XP_014494232.1|	4	6	Poly [ADP-ribose] polymerase 3	0.00057	0.00163	**0.34738**
gi|951067792|ref|XP_014524384.1|	1	5	Seed biotin-containing protein SBP65-like	0.00056	0.00335	**0.16606**
gi|950929466|ref|XP_014502187.1|	3	8	TSC22 domain family protein 1-like	0.00034	0.00159	**0.21445**

Table 4. Inferred identity and relative abundance of those proteins classified as common for MDF and MRF. NSAF ratios of MDF/MRF that are statistically significant ($p < 0.05$) are highlighted in bold

Identifier	SC		Matching protein description	NSAF		Ratio
	MDF	MRF	[*Vigna radiata* var. *radiata*]	MDF	MRF	MDF/MRF
gi|951066354|ref|XP_014523937.1|	557	500	Beta-conglycinin, beta chain-like isoform X1	0.09280	0.12154	0.76354
gi|951067727|ref|XP_014524354.1|	546	475	Beta-conglycinin, beta chain-like	0.09154	0.11597	0.78930
gi|951066306|ref|XP_014523923.1|	405	258	Beta-conglycinin, beta chain-like	0.08162	0.06543	1.24749
gi|951002540|ref|XP_014507363.1|	334	309	Beta-conglycinin, beta chain-like	0.05905	0.07935	0.74414
gi|950940165|ref|XP_014492536.1|	307	298	Beta-conglycinin, beta chain-like	0.05385	0.07568	**0.71145**
gi|951033982|ref|XP_014515878.1|	252	193	Beta-conglycinin, beta chain-like	0.04222	0.04778	0.88347
gi|951056419|ref|XP_014521758.1|	336	225	Glycinin G4-like	0.04168	0.04085	1.02034
gi|951066351|ref|XP_014523936.1|	261	172	Beta-conglycinin, alpha~ chain-like	0.03691	0.03480	1.06069
gi|951023258|ref|XP_014513134.1|	83	70	Albumin-2-like	0.02838	0.03518	0.80683
gi|951066358|ref|XP_014523938.1|	152	287	Beta-conglycinin, beta chain-like isoform X2	0.02524	0.06054	0.41695
gi|950930231|ref|XP_014503883.1|	71	44	Dehydrin DHN3-like	0.02324	0.02101	1.10658
gi|950951134|ref|XP_014495577.1|	66	29	Embryonic protein DC-8-like	0.01722	0.01126	1.52972
gi|950951230|ref|XP_014495608.1|	22	11	18 kDa seed maturation protein-like	0.01709	0.01149	1.48757
gi|951066718|ref|XP_014524029.1|	42	20	P24 oleosin isoform B	0.01632	0.01160	1.40759
gi|951034870|ref|XP_014516158.1|	19	17	Protein SLE2	0.01554	0.01962	0.79206
gi|951005658|ref|XP_014508213.1|	61	9	Low quality protein: late embryogenesis abundant protein D-29	0.01230	0.00259	4.75216
gi|951042174|ref|XP_014518107.1|	66	51	Basic 7S globulin 2-like	0.01228	0.01387	0.88571
gi|951067725|ref|XP_014524353.1|	39	38	Beta-conglycinin, beta chain-like, partial	0.01217	0.01699	**0.71650**
gi|950959908|ref|XP_014497548.1|	15	7	Protein SLE1 isoform X1	0.01105	0.00761	**1.45160**
gi|951016290|ref|XP_014511078.1|	67	16	Embryonic protein DC-8-like	0.01065	0.00374	**2.84737**
gi|951072910|ref|XP_014491941.1|	14	10	Non-specific lipid-transfer protein 1	0.00994	0.01083	0.91830
gi|951032441|ref|XP_014515393.1|	18	13	Desiccation protectant protein Lea14 homolog	0.00963	0.01016	0.94812
gi|951006474|ref|XP_014508481.1|	23	8	1-Cys peroxiredoxin	0.00816	0.00467	1.74620
gi|950968931|ref|XP_014499690.1|	86	26	Seed linoleate 9S-lipoxygenase-3	0.00767	0.00336	**2.28334**
gi|951066347|ref|XP_014523935.1|	22	21	Late embryogenesis abundant protein D-34-like	0.00753	0.01026	0.73413
gi|950974150|ref|XP_014500866.1|	8	5	Uncharacterised protein LOC106761813	0.00750	0.00651	1.15148
gi|950973966|ref|XP_014500828.1|	6	4	Uncharacterised protein LOC106761773	0.00740	0.00768	0.96356
gi|951006538|ref|XP_014508498.1|	11	2	Uncharacterised protein LOC106768046	0.00695	0.00186	**3.74767**
gi|951021491|ref|XP_014512682.1|	44	45	Sucrose-binding protein-like	0.00661	0.01016	0.64993
gi|951056290|ref|XP_014521723.1|	21	10	Uncharacterised protein LOC106778296	0.00657	0.00454	1.44907
gi|951000293|ref|XP_014506761.1|	23	10	Glucose and ribitol dehydrogenase homolog 1-like	0.00651	0.00408	1.59331
gi|950985610|ref|XP_014503380.1|	7	2	Uncharacterised protein LOC106763730	0.00592	0.00262	**2.25777**
gi|951035730|ref|XP_014516424.1|	10	6	Nucleoside diphosphate kinase 1	0.00550	0.00481	1.14333
gi|950986379|ref|XP_014503555.1|	10	6	17.5 kDa class I heat shock protein-like	0.00512	0.00438	1.16784
gi|951023922|ref|XP_014513287.1|	18	1	Glucose and ribitol dehydrogenase homolog 1-like	0.00511	0.00055	**9.27724**
gi|951040313|ref|XP_014517640.1|	17	9	Glucose and ribitol dehydrogenase homolog 1-like	0.00481	0.00350	1.37506
gi|950969621|ref|XP_014499874.1|	6	3	Late embryogenesis abundant protein 2	0.00472	0.00379	1.24558
gi|951014217|ref|XP_014510496.1|	36	3	Heat shock 70 kDa protein	0.00447	0.00058	7.64503
gi|950950919|ref|XP_014495514.1|	8	1	18 kDa seed maturation protein-like	0.00417	0.00112	3.72353
gi|950993033|ref|XP_014504815.1|	20	3	Alcohol dehydrogenase 1-like	0.00416	0.00103	4.02609
gi|951048093|ref|XP_014519608.1|	35	18	Canavalin	0.00397	0.00275	1.44546
gi|951022780|ref|XP_014513011.1|	12	5	Peroxygenase [Vigna radiata var. radiata]	0.00389	0.00274	1.41912
gi|950943234|ref|XP_014493768.1|	8	4	Peptidyl-prolyl cis-trans isomerase 1	0.00383	0.00335	1.14194
gi|951023386|ref|XP_014513168.1|	10	6	Late embryogenesis abundant protein D-34-like isoform X1	0.00327	0.00308	1.06164
gi|951039676|ref|XP_014517471.1|	9	1	Late embryogenesis abundant protein 2-like	0.00324	0.00056	**5.80070**
gi|950968907|ref|XP_014499686.1|	36	14	Seed linoleate 9S-lipoxygenase-2	0.00322	0.00180	**1.79275**
gi|951023264|ref|XP_014513135.1|	10	3	Uncharacterised protein LOC106771650	0.00317	0.00204	1.55016
gi|951040842|ref|XP_014517782.1|	5	6	40S ribosomal protein S14	0.00308	0.00466	0.66011

Identifier	SC		Matching protein description	NSAF		Ratio
	MDF	MRF	[*Vigna radiata* var. *radiata*]	MDF	MRF	MDF/MRF
gi|951023134|ref|XP_014513100.1|	6	2	UPF0098 protein TC_0109-like	0.00306	0.00194	1.57800
gi|951065326|ref|XP_014523717.1|	18	2	Luminal-binding protein, partial	0.00303	0.00045	6.77394
gi|951066326|ref|XP_014523928.1|	23	24	Beta-conglycinin, beta chain-like	0.00294	0.00447	0.65718
gi|950925699|ref|XP_014494982.1|	14	1	Formate dehydrogenase 1, mitochondrial-like isoform X1	0.00279	0.00036	7.69934
gi|950973879|ref|XP_014500811.1|	6	4	Late embryogenesis abundant protein D-34-like	0.00274	0.00301	0.91121
gi|951042601|ref|XP_014518193.1|	6	4	Uncharacterised GPI-anchored protein At5g19250-like	0.00243	0.00283	0.85912
gi|950994560|ref|XP_014505167.1|	13	1	UTP--glucose-1-phosphate uridylyltransferase	0.00233	0.00030	7.77599
gi|950974705|ref|XP_014500967.1|	22	9	Nudix hydrolase 3-like	0.00233	0.00155	1.50251
gi|950933283|ref|XP_014511756.1|	28	8	Alpha-1,4 glucan phosphorylase L isozyme	0.00222	0.00097	**2.28607**
gi|950951948|ref|XP_014495815.1|	17	1	Heat shock cognate 70 kDa protein 2	0.00215	0.00025	8.59277
gi|950933402|ref|XP_014511960.1|	12	5	Elongation factor 1-alpha	0.00212	0.00146	1.45311
gi|951036864|ref|XP_014516740.1|	6	1	40S ribosomal protein S6-like	0.00208	0.00093	2.22807
gi|950993253|ref|XP_014504861.1|	9	3	Fructose-bisphosphate aldolase, cytoplasmic isozyme	0.00205	0.00140	1.46107
gi|950954866|ref|XP_014496519.1|	5	5	40S ribosomal protein S8-like	0.00193	0.00272	0.71008
gi|950944995|ref|XP_014494154.1|	7	1	Malate dehydrogenase, mitochondrial	0.00171	0.00064	**2.68187**
gi|951021073|ref|XP_014512575.1|	13	4	Granule-bound starch synthase 1, chloroplastic/amyloplastic-like	0.00169	0.00091	1.85532
gi|323149044|ref|YP_004222824.1|	10	2	ATPase subunit 1 (mitochondrion)	0.00164	0.00061	**2.68466**
gi|950933029|ref|XP_014511428.1|	7	2	Actin-1-like	0.00155	0.00081	1.91189
gi|950934982|ref|XP_014515635.1|	13	1	Heat shock protein 83	0.00148	0.00020	**7.54676**
gi|951072874|ref|XP_014491920.1|	7	6	60S ribosomal protein L4	0.00145	0.00170	0.85444
gi|950979939|ref|XP_014501963.1|	8	2	Protein disulfide-isomerase-like	0.00119	0.00044	2.72129
gi|950929654|ref|XP_014502725.1|	3	5	40S ribosomal protein S3-1-like	0.00114	0.00295	0.38647
gi|951028515|ref|XP_014514444.1|	6	5	Serine carboxypeptidase-like	0.00100	0.00103	0.97390
gi|951027555|ref|XP_014514203.1|	6	3	ATP synthase subunit beta, mitochondrial	0.00099	0.00066	1.50343
gi|950945335|ref|XP_014494232.1|	9	1	Poly [ADP-ribose] polymerase 3	0.00086	0.00028	3.13469
gi|950929466|ref|XP_014502187.1|	11	3	TSC22 domain family protein 1-like	0.00081	0.00034	2.41113
gi|950975966|ref|XP_014501194.1|	5	1	Elongation factor 2	0.00053	0.00028	1.88891

4. Conclusion

The main objectives of this study were to expand our knowledge and understanding of the effect of specific processing conditions on nutritional composition of mungbean flours, as well as providing significant and comprehensive analyses of mungbean protein composition and relative abundance using a comparative proteomic approach. Processing methods used in this study imparted significant changes to mungbean nutritional composition, leading to altered functionality and potential end-use applications. Investigating the effect of processing conditions on protein composition and relative abundance is important for the production of functional, value-added high protein fractions for food applications. Innovative processing methods applied to mungbean and other pulse flours, combined with advanced proteomic tools for characterising protein composition and relative abundance, will provide an effective platform for developing concentrated protein flours and isolates. This study paves the way for further work focussing on the production of functional flours with enhanced digestibility and bioavailability of nutrients. Enhanced nutritional qualities and promotion of the health benefits of pulse products could potentially lead to wider consumer acceptance and increased sustainability for future food production.

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Appendix A

Flour specific proteins identified from a comparison of MWF and MDF

Identifier	SC	Matching protein description [*Vigna radiata* var. *radiata*]	NSAF
MDF Specific			
gi|951066306|ref|XP_014523923.1|	405	Beta-conglycinin, beta chain-like	0.08168
gi|951006538|ref|XP_014508498.1|	11	Uncharacterised protein LOC106768046	0.00696
gi|951004779|ref|XP_014507957.1|	5	Uncharacterised protein LOC106767550	0.00372
gi|951042601|ref|XP_014518193.1|	6	Uncharacterised GPI-anchored protein At5g19250-like	0.00243
gi|950966752|ref|XP_014499164.1|	5	Translationally-controlled tumor protein homolog	0.00236
gi|951033740|ref|XP_014515808.1|	19	Heat shock 70 kDa protein-like	0.00235
gi|950971152|ref|XP_014500243.1|	9	Annexin-like protein RJ4	0.00229
gi|950933402|ref|XP_014511960.1|	12	Elongation factor 1-alpha	0.00212
gi|951036864|ref|XP_014516740.1|	6	40S ribosomal protein S6-like	0.00208
gi|950943809|ref|XP_014493887.1|	15	Heat shock cognate 70 kDa protein 2	0.00189
gi|950944995|ref|XP_014494154.1|	7	Malate dehydrogenase, mitochondrial	0.00172
gi|950992302|ref|XP_014504685.1|	13	Heat shock cognate protein 80	0.00149
gi|950994103|ref|XP_014505054.1|	6	Glucose-1-phosphate adenylyltransferase small subunit 2, chloroplastic	0.00100
gi|950953641|ref|XP_014496230.1|	5	Enolase	0.00100
gi|951071101|ref|XP_014491069.1|	5	Uncharacterised protein LOC106753730	0.00058
gi|951062968|ref|XP_014523173.1|	7	Alpha-glucan water dikinase, chloroplastic	0.00038

Appendix B

Flour specific proteins identified from a comparison of MGF and MWF

Identifier	SC	Matching protein description [*Vigna radiata* var. *radiata*]	NSAF
MGF Specific			
gi|951066306|ref|XP_014523923.1|	243	Beta-conglycinin, beta chain-like	0.06115
gi|951008854|ref|XP_014509123.1|	8	Pathogenesis-related protein 2-like	0.00585
gi|951006538|ref|XP_014508498.1|	7	Uncharacterised protein LOC106768046	0.00568
gi|951072921|ref|XP_014491947.1|	5	Non-specific lipid-transfer protein 1-like	0.00505
gi|951001085|ref|XP_014506982.1|	6	Pathogenesis-related protein 2-like	0.00464
gi|950971152|ref|XP_014500243.1|	12	Annexin-like protein RJ4	0.00400
gi|951036864|ref|XP_014516740.1|	6	40S ribosomal protein S6-like	0.00265
gi|951068867|ref|XP_014489923.1|	9	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic-like	0.00243
gi|950992302|ref|XP_014504685.1|	17	Heat shock cognate protein 80	0.00242
gi|950944995|ref|XP_014494154.1|	7	Malate dehydrogenase, mitochondrial	0.00242
gi|950956929|ref|XP_014496954.1|	15	Heat shock cognate 70 kDa protein 2-like	0.00237
gi|951011572|ref|XP_014509821.1|	5	Annexin-like protein RJ4	0.00172
gi|951057234|ref|XP_014521938.1|	5	Fructokinase-2-like	0.00165
gi|950953641|ref|XP_014496230.1|	7	Enolase	0.00162
gi|950933402|ref|XP_014511960.1|	7	Elongation factor 1-alpha	0.00154
gi|951034199|ref|XP_014515942.1|	12	Linoleate 9S-lipoxygenase-like	0.00145
MWF Specific			
gi|950973966|ref|XP_014500828.1|	5	Uncharacterised protein LOC106761773	0.01686
gi|950959908|ref|XP_014497548.1|	6	Protein SLE1 isoform X1	0.01158

Appendix C

Flour specific proteins identified from comparison of MDF and MRF

Identifier	SC	Matching protein description [*Vigna radiata* var. *radiata*]	NSAF
MDF Specific			
gi|951004779|ref|XP_014507957.1|	5	Uncharacterised protein LOC106767550	0.00372
gi|950966752|ref|XP_014499164.1|	5	Translationally-controlled tumor protein homolog	0.00236
gi|951033740|ref|XP_014515808.1|	19	Heat shock 70 kDa protein-like	0.00235
gi|950971152|ref|XP_014500243.1|	9	Annexin-like protein RJ4	0.00229
gi|950943809|ref|XP_014493887.1|	15	Heat shock cognate 70 kDa protein 2	0.00189
gi|950992302|ref|XP_014504685.1|	13	Heat shock cognate protein 80	0.00149
gi|951067792|ref|XP_014524384.1|	6	Seed biotin-containing protein SBP65-like	0.00141
gi|950994103|ref|XP_014505054.1|	6	Glucose-1-phosphate adenylyltransferase small subunit 2, chloroplastic	0.00100
gi|950953641|ref|XP_014496230.1|	5	Enolase	0.00099
gi|951071101|ref|XP_014491069.1|	5	Uncharacterised protein LOC106753730	0.00058
gi|950963716|ref|XP_014498402.1|	5	Cell division cycle protein 48 homolog	0.00049
gi|951062968|ref|XP_014523173.1|	7	Alpha-glucan water dikinase, chloroplastic	0.00038