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Original Research Article

Proximate composition and anti-nutritional factors of fava-bean (*Vicia faba*), green-pea and yellow-pea (*Pisum sativum*) flour

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ABSTRACT

Pulse grains were identified as a key resource for food innovation during the International Year of the Pulse (IYP), 2016. Pulse flour offers a sustainable source of plant protein for innovation in protein enriched cereal based foods. Fava-bean (*Vicia faba*), green- and yellow-pea (*Pisum sativum*) flour were analysed for proximate composition, minerals, amino acids, phenolic content, phytic acid and trypsin inhibitory activity. Fava-bean flour had the highest protein content (28 g/100 g), while green-pea flour had the highest total dietary fibre content (15 g/100 g). All three flours contained essential amino acids in adequate quantity, highlighting them as a source of good quality protein for in the formulation of protein-enriched foods. Fava-bean flour had significantly higher phenolic content and antioxidant activity than pea flours (387 mg GAE/100 g and 250 mg AAE/100 g respectively). Pulse flour contained high levels of potassium and zinc, while fava-bean flour was also high in iron. Phytic acid ranged from 543 to 889 mg/100 g; the lowest of which was observed in green-pea flour. Green-pea flour also exhibited the lowest trypsin inhibition (3.7 TIU/mg). Results demonstrate the significant potential of pulse flour to enhance the nutritional value of cereal based foods which is not possible with wheat flour alone.

1. Introduction

Consumer health has become a key focus for the food industry as well as for consumers. As highlighted by Go et al. (2014), the role of nutrition on the prevalence of cardiovascular disease, diabetes and obesity, has been well documented. Over the last two decades, consumers have begun to accept that foods directly contribute to their health and are now seeking nutritionally enhanced products in almost all aisles of the supermarket (Bigliardi and Galati, 2013). As protein consumption has been steadily increasing, consumption patterns of protein are also changing. There is an increasing drive toward plant based proteins, whereby protein enriched foods have moved beyond dairy and meat to a variety of other food sectors.

Pulses are leguminous plants which are harvested solely for their dry seeds. Unlike other legumes such as soybean and groundnut, pulse grains, including beans, chickpeas, lentils and peas, are characterised by a low fat content. While in tropical and sub-tropical countries they are the second most important source of protein after cereals, they remain largely under-utilised as a source of protein in Western countries (Pasiakos et al., 2015). Soybean remains the most widely consumed legume as a source of plant protein. However, recent concerns regarding genetically modified crops as well as allergen and intolerance issues have begun to drive demand for other legumes and pulses (Giménez et al., 2012; Felgate, 2015). To encourage a new focus on pulses as sustainable and nutritionally important crops, the United Nations declared 2016 as the International Year of the Pulse, promoting them as “nutritious seeds for a sustainable future”.

Pulse flour offers a versatile and highly nutritious source of protein for healthy food innovation. As with cereals, starch is a primary component of pulse flour making it suitable for use in nutritionally enhanced breads, pastas and other bakery products (Giménez et al., 2012; Zafar et al., 2015; Herranz et al., 2016). Pulse flour can provide high levels of good quality protein, resistant starch and dietary fibres (Day, 2013; Messina, 2014). While plant proteins have lower biological value than animal proteins, due to their low levels of sulphur-containing amino acids, pulses are a rich source of several other essential amino acids including lysine, leucine, isoleucine and phenylalanine. When combined with other grains such as wheat and rice, pulses including

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peas and beans can provide a well-balanced essential amino acid profile (Boye et al., 2010a). Pulse flour is also an important source of micronutrients, including vitamins and minerals, in particular iron, zinc, folate and other B vitamins, and phytochemicals (Campos-Vega et al., 2010). As such, pulse flour offers an alternative approach to increasing the consumption of beneficial micronutrients through food innovation and re-formulation.

Other minor components of pulse flour include proteins such as lectins and enzyme inhibitors, and phytic acid. While these have previously been considered anti-nutritional due to their ability to bind micronutrients and reduce macronutrient digestibility, some studies also show potential health benefits of including low levels of such compounds in the diet (Campos-Vega et al., 2010). As highlighted by Amarakoon et al. (2012), the correct ratio of anti-nutrients to nutrients can reduce the negative impact on digestibility and play a beneficial role in cellular processes including antioxidant and anti-inflammatory activities.

Research into the potential applications for chickpea and lentil flour has been growing due to their wider consumption in Western populations (Han et al., 2010; Zafar et al., 2015). While fava-bean and pea flour have been under utilised in human nutrition and grown mainly for animal feed (Laudadio et al., 2012; Messina, 2014; Tufarelli and Laudadio, 2015; Koivunen et al., 2016), interest in their food application is now increasing. This is somewhat due to their environmental benefits including reduced energy requirements, nitrogen fixation and their high production yield in temperate climates (Vollmann, 2016; Tulbek et al., 2017). Fava-bean flour has been used with wheat flour in pasta re-formulations, while pea flours have been used for the re-formulation of breads and crackers, demonstrating their potential in the manufacture of protein-enhanced products (Giménez et al., 2012; Mondor et al., 2014; Turco et al., 2016; Millar et al., 2017). Pulse flour is also naturally gluten-free and may also have numerous applications in enhancing the nutritional value of gluten-free foods such as pasta, breads and snacks (Han et al., 2010; Laleg et al., 2016).

The objectives of the current study were to evaluate the nutritional quality of fava-bean, green-and yellow-pea flour, and assess their potential to substitute or complement wheat flour in new food formulation, particularly in relation to current nutritional guidelines and dietary requirements.

2. Materials and methods

2.1. Materials

Split fava beans (*Vicia fava,* cv. Victor), split yellow peas (*Pisum sativum* L.) and split green peas (*Pisum sativum,* cv. Large blue) were purchased from Hodmedod Ltd (Suffolk, United Kingdom): Pulses were milled using a Perten Lab mill 3100 (Perten, Australia), equipped with a 0.5–1.00 mm sieve screen for a flour particle size range of 500–900 μm. Commercial strong-wheat flour was obtained from Shackelton's Milling (Co. Meath, Ireland) and analysed as a reference flour.

All reagents used were of analytical grade. Acetone, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), iron chloride (FeCl3), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), gallic acid, ascorbic acid and 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Fisher Scientific (Fisher Scientific Ireland, Dublin, Ireland). Benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPNA) and all other chemicals were purchased from Sigma Aldrich (Wicklow, Ireland). Enzymes (trypsin, protease, amylase and amyloglucosidase) were purchased from Megazyme (Bray, Ireland).

2.2. Proximate composition

Moisture content of the flours was determined using the AACC method 14–15 A (AACC, 2001). Flour samples were weighed (10 g) and dried using a Brabender oven at 130 °C for 1 h. Ash content was determined using AACC method 08-01.01 (AACC, 1981). Total nitrogen was determined by the combustion method based on the Dumas principle using a nitrogen analyzer (FP-328 Leco Instrument, Leco Corporation, USA). Combustion of the samples (200 ± 2 mg) took place in a sealed furnace at 1150 °C. Nitrogen to protein conversion factor of 5.70 was used to calculate total protein. Fat content was determined using the AOAC acid hydrolysis method, 922.06 (AOAC, 2005) using the ANKOM HCl Hydrolysis System and the ANKOM Extractor (ANKOM Technology, New York, USA). Total carbohydrate was calculated by difference (100 - sum of protein, fat, ash and moisture). Soluble (SDF) and insoluble (IDF) dietary fibre was determined according to AOAC Method 991.43, (AOAC, 1995) using the ANKOM dietary fibre analyser (ANKOM technology, New York, USA). Results were corrected for moisture content and reported on a g/100 g dry matter (DM) basis. The % energy provided by protein was calculated using the equation below:

$$\%\text{ energy from protein} = \frac{\text{Grams of protein per 100 g x Conversion factor for protein}}{\text{Total energy per 100 g}} \text{ x } \frac{100}{1} \quad (1)$$

Where, protein and carbohydrate provide 4 kcal/g and fat provides 9 kcal/g (Food Safety Authority of Ireland, 2016).

2.3. Amino acid analysis

Total amino acids were determined according to the amino acid hydrolysis compendium method (AOAC, 2000). Proteins were hydrolysed in 6 M HCl at 110 °C for 23 h using a Glas-Col combo mantle (Gals-Col, Terre Haute, USA) for the determination of all amino acids except sulphur amino acids and tryptophan. Methionine and cysteine were oxidized with performic acid to methionine sulphone and cysteic acid, respectively, and then hydrolysed with HCl. Lysozyme was used as a control for hydrolysis. The resulting hydrolysates were diluted 1 in 2 with the internal standard, norleucine, to give a final concentration of 125 nmol/ml. Amino acids were quantified using a Jeol JLC-500/V amino acid analyser (Jeol (UK) Ltd., Garden city, Herts, UK) fitted with a Jeol Na^+ high performance cation exchange column. Analysis was carried out in a single replicate and an amino acid standard containing all amino acids detectable in the system was run with the samples.

2.4. Mineral analysis

Mineral content of the samples was analysed by atomic absorption spectroscopy on previously ashed samples. Analyses were performed on a 3110 Perkin-Elmer Atomic Absorption Spectrophotometer (The Perkin-Elmer Corporation, Norwalk, USA) with standard conditions for each of the elements as described by the instrument manufacturer (Perkin-Elmer, 1994).

2.5. Phytochemical composition

2.5.1. Determination of total phenolic content and antioxidant activity

Quantification of total phenolic content (TPC) and antioxidant activity (AOX) was carried out according to Rajauria et al. (2010) with some modifications. Briefly, 1 g of flour was extracted in 10 ml of acetone (80%) for 1 h at 40 °C in a rotary incubator. Samples were centrifuged at 10,000 rpm for 10 min and the supernatant was collected. The residue was washed with fresh solvent and re-extracted twice more. The supernatants were pooled and concentrated using a rotary evaporator at 40 °C. The samples were adjusted to 10 ml with deionised water and stored in the dark at −20 °C until analysis. TPC was measured according to the Folin-Ciocalteu method as outlined by Cox et al. (2010). Results were corrected for moisture and expressed as mg gallic acid equivalents (GAE) per 100 g dry matter. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays were carried out according to Rajauria et al. (2010). DPPH radical scavenging activity was calculated

Table 1
Proximate composition of fava-bean, green- and yellow-pea, and wheat flour (g/100 g DM).

	Protein	Ash	Fat	Moisture content (%)	Total dietary fibre	Insoluble dietary fibre	Soluble dietary fibre
Fava bean	27.99 ± 0.06^{a}	3.40 ± 0.09^{a}	1.57 ± 0.11^{a}	12.30 ± 0^{c}	13.80 ± 0.97^{a}	9.07 ± 0.56^{a}	4.74 ± 0.40^{a}
Green pea	21.50 ± 0.08^{c}	2.76 ± 0.06^{a}	1.28 ± 0.09^{b}	10.60 ± 0^{d}	15.58 ± 1.31^{a}	10.88 ± 1.02^{a}	4.92 ± 0.51^{a}
Yellow pea	22.33 ± 0.05^{b}	3.52 ± 0.80^{a}	1.40 ± 0.04^{ab}	13.35 ± 0.1^{a}	14.84 ± 0.93^{a}	9.77 ± 0.68^{a}	5.08 ± 0.50^{a}
Wheat	12.81 ± 0.06^{d}	0.96 ± 0.19^{b}	1.53 ± 0.08^{a}	12.70 ± 0^{b}	10.08 ± 1.20^{b}	4.93 ± 0.23^{b}	5.15 ± 0.99^{a}

Data presented as means ± standard deviation from triplicate analysis.
a–d Values followed by different superscripts in the same column are significantly different ($p < 0.05$).

using the following equation:

$$\%\,\text{radical scavenging activity} = \frac{\text{control abs-test abs}}{\text{control abs}} \times 100 \quad (2)$$

Results were corrected for moisture and expressed as mg ascorbic acid equivalents (AAE) and mg trolox equivalent (TE) per 100 g dry matter for DPPH and FRAP assays respectively.

2.5.2. Phytic acid content

Phytic acid content of flour samples was determined using Megazyme Phytate/Total Phosphorus kit (K-PHYT 11/15) (Megazyme, Bray, Ireland). Phosphorus released by phytase and alkaline phosphatase was determined colorimetrically. Phytic acid content was calculated according to manufacturer's instructions and reported as mg/100 g of dry matter.

2.5.3. Trypsin inhibitory activity

2.5.3.1. Reagent preparation. Trypsin inhibitory activity was carried out according to Kakade et al. (1974) with some modifications. Tris-buffer (0.05 M) was prepared with 2.94 g of $CaCl_2.H_2O$ and 6.05 g tris (hydroxymethyl) aminomethane in 900 ml distilled water. The pH was adjusted to 8.2 and the volume was brought up to 1 l with distilled water. Substrate solution was prepared by dissolving 80 mg BAPNA (benzoyl-DL-arginine-*p*-nitroanilide hydrochloride) in 2 ml of DMSO and diluted to 200 ml with Tris buffer heated to 37 °C. The substrate solution was prepared fresh daily and kept at 37 °C while in use. Standard trypsin stock solution was prepared by dissolving 0.5 g porcine pancreas trypsin in 500 ml HCl (0.001 M) and kept at 4 °C. A working solution of 185 mg/l was prepared daily. When subjected to the assay procedure, 2 ml of the standard gives an absorbance value of 0.410 ± 0.010.

2.5.3.2. Procedure. One gram of flour was extracted with 50 ml NaOH (0.01 M) for 1 h at room temperature. The pH was monitored during extraction to be kept between 8.4 and 10.0. Extracts were diluted 1:10 to give trypsin inhibition of 30–70% (Liu and Markakis, 1989). To a test tube, 2 ml trypsin solution was added to 1 ml diluted extract and 1 ml distilled water. The tubes were placed in a shaking water bath set to 37 °C. After 10 min, 5 ml BAPNA solution was added to each test tube which was vortexed for 10 s. The samples were incubated for 10 min after which 1 ml acetic acid (30%) was added to each tube to terminate the reaction. A reagent blank was prepared by adding 1 ml acetic acid to a test tube containing 2 ml trypsin and 2 ml distilled water before adding 5 ml of BAPNA solution. The absorbance due to the release of *p*-nitroaniline was determined at 410 nm. Trypsin inhibitory activity (TIA) was expressed in units of trypsin inhibited (TIU) per mg of dry matter (Kakade et al., 1974).

2.6. Statistical analysis

All analyses were performed in triplicate, unless expressly stated otherwise. Results were expressed as mean values ± standard deviations. Analysis of variance (ANOVA) was carried out using SAS (Statistical Analysis System version 9.4, USA). Statistical significance was considered at $p < 0.05$. Where ANOVA indicated significant differences were present, Tukey's pairwise comparison was conducted to identify where sample differences occurred. To identify relationships between total polyphenols and antioxidant activity bivariate Pearson's correlation analysis was carried out.

3. Results and discussion

3.1. Proximate composition

The proximate composition of wheat, fava-bean, green-pea and yellow-pea flour is presented in Table 1. Moisture content varied significantly across wheat and pulse flour, from 10.6% to 12.7%. This is comparable to those previously reported for pea and bean flour (Petitot et al., 2010; Turco et al., 2016). Total ash content was significantly higher for all pulse flours (2.8 to 3.5 g/100 g) compared with wheat flour (1 g/100 g). The higher ash content of pulse flour means they have the potential to increase mineral intake in the diet when combined in food products with low ash flour such as wheat. However, high ash flour (> 1%) is generally considered poorer quality by bakers due to the dilution effect of the functional proteins (Cauvain, 2015). This can have negative effect in bread-making and may result in reduced loaf volume (Mohammed et al., 2014). Plant-based emulsifiers may assist in improving such organoleptic properties (Mastromatteo et al., 2015), while in other applications such as pastas, crackers or biscuits, sensory acceptability may be higher (Han et al., 2010; Zucco et al., 2011; Giménez et al., 2015; Millar et al., 2017).

Fat content was less than 2% for all pulse flour. This offers an advantage over chickpea flour which has been reported to range from 6 to 7% fat (De Almeida Costa et al., 2006; Thushan Sanjeewa et al., 2010), and soy flour which contains ~20% fat (Day, 2013). The presence of these higher levels of fat, particularly in soy flour, can be a barrier to incorporating them into foods due to the lipoxygenase-catalysed oxidation of unsaturated fatty acids to volatile compounds, affecting both the flavour and shelf-life of some food products (Guichard, 2002).

Total carbohydrate content was lower in all pulse flours (54.7–63.9 g/100 g) compared with wheat flour (72 g/100 g). Berrios et al. (2010) reported similar carbohydrate content in lentils and chickpeas (62.49–65.7 g/100 g). Total dietary fibre (TDF) was significantly higher in pulse flour ($p < 0.05$). Pulse flour contained between 13.8 and 16.6 g/100 g TDF, compared with 10.1 g/100 g in wheat flour. TDF was higher than that reported by Turco et al. (2016) and Petitot et al. (2010) in fava-bean flour (6.4 and 7.3 g/100 g respectively). The current recommendation as outlined by the European Food Safety Authority (EFSA) is for consumption of > 25 g fibre a day to maintain normal bowel function, aid in weight management and reduce the risks of coronary heart diseases and type 2 diabetes (EFSA Panel on Dietetic Products, N.a.A., 2010). It is estimated that only 20% of people meet this recommendation however (European Commission, 2019). The higher content of TDF observed in all three pulse flours, was due to the significantly higher content of insoluble dietary fibre (IDF) compared with wheat flour. Where soluble fibre (SDF) is readily soluble in water, and forms viscous solutions, IDF is not readily soluble in water, and passes undigested to the large intestine. It should be noted however that the EFSA Panel on Dietetic Products, N.a.A. (2010) reported that not all dietary fibres could be easily classified by this method as some soluble fibres do not form viscous solutions, and solubility does not always predict the physiological effects. The ability of SDF to form viscous solutions is reported to slow down gastric emptying which reduces the absorption of glucose and triglycerides across the intestine (Kaczmarczyk et al., 2012). Dietary fibre that does not form viscous solutions and is passed undigested to the large intestine, including resistant starches and oligosaccharides, increases stool bulk and decreases intestinal transit time. This maintains normal bowel function and can prevent gastrointestinal disorders (EFSA Panel on Dietetic Products, N.a.A., 2010). The role of IDF in weight management is also due their role in adding bulk to the diet, inducing satiety and their lower energy density (Lattimer and Haub, 2010). It was reported by Kendall et al. (2010) that there is also some evidence to suggest IDF may aid in the prevention of colorectal cancers. However, the authors highlighted inconsistencies in the research published to date, and that further rigorous population studies are required to establish a link. For this reason, EFSA avoided making recommendations on fibre intake for the prevention of colorectal cancers in their 2010 report and the situation has not changed since. However, the United States Food and Drug Administration (FDA) do have an approved health claim relating to dietary fibre, stating that increased consumption of dietary fibre may reduce some types of cancer when combined with a decreased consumption of fats (FDA, 2008). Lattimer and Haub (2010) suggested that this preventative effect may be a result of increased bile acid excretion, increased short chain fatty acid production, carcinogen binding effects, increased antioxidants, and increased vitamin and minerals intake. It had been previously believed that IDF solely contributed to the physiological effects previously mentioned; however Kaczmarczyk et al. (2012) highlighted several studies that demonstrated the role of IDF in improving insulin sensitivity, thus also potentially contributing to the prevention of type 2 diabetes.

All three pulse flours had significantly higher protein content compared with wheat flour ($p < 0.05$). Fava-bean flour had the highest protein content, 27.99 g/100 g (Table 1), and conversely, the lowest carbohydrate content, 54.7 g/100 g ($p < 0.05$). The protein content observed in this study was comparable to that previously reported in beans and peas. Masey O'Neill et al. (2012) reported 22–24.6 g/100 g and 19.5–22.4 g/100 g in several different cultivars of fava bean and peas. Koivunen et al. (2016) reported 31.8 g/100 g in fava-bean flour (cv. Kontu). The protein content was also comparable to that of other more commonly consumed pulse flour including lentil and chickpea flours (De Almeida Costa et al., 2006; Chung et al., 2008; Boye et al., 2010b; Fares and Menga, 2012). Soy flour has a higher content of protein compared with most pulses ranging from 32 to 43 g/100 g (Pisulewska and Pisulewski, 2000; Masey O'Neill et al., 2012). This can also bring challenges in food product development however. It can increase the interactions of proteins with saponins and volatile flavour compounds within the food matrix, leading to the production of unpleasant beany flavours (Guichard, 2002; Heng et al., 2004).

The percent contribution of protein to total energy of the flour was 32.5, 24.4, 26.3 and 14.5 for fava-bean, green- and yellow-pea and wheat flour respectively. High protein foods are those where at least 20% of the total energy is provided by protein (Food Safety Authority of Ireland, 2016). All three pulse flours had more than the required 20% of energy from protein to meet the "high protein" claim. Current intake of protein in Europe is estimated at 12–20% of overall energy intake (European Food Safety Authority, 2017). However, as Barton (2014) highlighted in a report relating to plant proteins, there is a growing body of evidence to suggest small increases in protein consumption may reduce the risk of developing symptoms of metabolic syndrome. Although there is international variation, globally wheat is the highest contributor to the calories and protein consumed than any other foodstuff (USDA-FAS, 2017). Therefore, supplementing wheat flour with pulse flour in the diet may increase the contribution of protein to overall energy intake. When combined with wheat flour, fava-bean flour can increase the protein content from 12.81 to 15.85 g/100 g at just 20% substitution, and up to 20.40 g/100 g following 50% substitution. Millar et al. (2017) increased the protein content of baked crackers from 8.36 g/100 g to 13.02 g/100 g following substitution of wheat flour with 40% fava-bean flour. The authors reported no significant effects on cracker texture and good sensory acceptability (> 7 in the 9-point hedonic scale). Following 10% substitution of wheat flour for malted yellow-pea flour in bread-making, Mondor et al. (2014) reported an increase in protein content from 13.5 to 14.5 g/100 g with no effect on loaf volume.

Bioavailability of proteins from plants and legumes is considered poor compared with animal proteins as they contain higher levels of poorly digestible protein fractions, along with a high content of antinutritional factors including enzyme inhibitors, phytates and tannins (Boye et al., 2010a). While peas and beans have been reported to have higher digestibility compared with soybean, largely as a result of their lower content of trypsin inhibitors (Sarwar Gilani et al., 2012), processing methods should be considered to further improve their digestibility and protein bioavailability. Xu et al. (2016) observed significant increases in protein digestibility of chickpea flour following several processing steps including soaking, pressure cooking and microwave cooking. The authors reported significant inverse correlations between decreases in tannin and phytate content and increase in *in vitro* protein digestibility of the flour following processing. Khattab and Arntfield (2009) also reported significant decreases in tannin and phytate content of several varieties of bean, pea and cowpea flour following boiling, roasting and microwave cooking.

3.2. Amino acids

The amino acid composition of the pulse and wheat flour is presented in Table 2. Pulse proteins are often considered to be poor quality protein due to an imbalance of essential amino acids, compared with animal proteins, which are a rich source of all essential amino acids. All flours had similar levels of total essential amino acids, ranging from

Table 2
Amino acid composition of fava-bean, green- and yellow-pea and wheat flour (mg/g protein).

	Fava bean	**Green** pea	**Yellow** pea	Wheat	mg/kg per day[A]	mg/g protein[B]
Essential amino acids						
Histidine	**32**[a]	**35**	**27**	21	10	15
Isoleucine	**36**	**35**	**38**	32	20	30
Leucine	71	70	77	78	39	59
Lysine	**55**	**63**	**70**	42	30	45
Methionine	9	11	12	23	10	16
Phenylalanine	**43**	**38**	**49**	31	25	38[C]
Threonine	34	**37**	**40**	34	15	23
Valine	39	39	49	51	26	10
Non-essential amino acids						
Alanine	**37**	**41**	**43**	36	–	–
Arginine	**77**	**74**	**79**	41	–	–
Aspartic acid	**102**	**109**	**114**	53	–	–
Cysteic acid	17	12	16	25	–	–
Cysteine	11	12	14	25	–	–
Glutamic acid	142	147	166	372	–	–
Glycine	34	**37**	**42**	36	–	–
Serine	43	43	47	52	–	–
Taurine	21	31	21	38	–	–
Tyrosine	27	21	30	31	–	–

a Figures in bold contain higher mg/g protein of that amino acid compared with wheat flour.
A Amino acid recommendations, mg/kg body mass per day for adults (WHO/FAO/UNU, 2007).
B Amino acid recommendations, mg/g protein consumed for adults (WHO/FAO/UNU, 2007).
C Phenylalanine and Tyrosine.

310 mg/g protein in wheat flour to 362 mg/g protein in yellow-pea flour. The profile of the amino acids differed however, with fava-bean and pea flours having a higher content of histidine, isoleucine, lysine and phenylalanine compared with wheat flour. Pulse flour was found to be lower in the sulphur-containing amino acids, methionine and cysteine, which are considered the limiting amino acids of pulse proteins. In comparison, where the wheat flour was rich in methionine and cysteine, it was lower in lysine, phenylalanine, arginine and aspartic acid. The amino acid content observed in fava-bean and pea flours was in agreement with those published by Koivunen et al. (2016) who reported 8 and 7 mg methionine/g protein and 16 and 13 mg cysteine/g protein in peas and fava beans respectively. Aspartic and glutamic acids were found in the highest quantity in all pulse flours. This was also in agreement with previously published data by Siddhuraju and Becker (2005); Boye et al. (2010b) and Koivunen et al. (2016). The total content of essential amino acids in soy flour was reported to be 386 mg/g protein (Masey O'Neill et al., 2012). The amino acid profile was found to be similar to fava-bean and pea flours, with leucine and lysine found in the highest amounts, 61 and 80 mg/g protein. The authors did, however, report a higher content of methionine (28 mg/g protein). Compared with proteins from animal sources such as whey protein, Teba et al. (2017) reported total essential amino acids of 396 mg/g protein. While methionine content was similarly low (8 mg/g protein), levels of isoleucine and leucine were much higher (60 and 107 mg/g protein). With regards to consumption of plant proteins however, the data in Table 2 highlights the potential benefits that may be achieved through complementary protein intake in the diet.

3.3. Mineral analysis

All three pulse flours had significantly higher content of zinc, magnesium, iron and potassium compared to wheat flour, $p < 0.05$ (Table 3). Fava-bean flour had the highest content of potassium, while yellow-pea flour had the highest content of magnesium. The results in this present study were comparable to those previously published by Cabrera et al. (2003) who reported 7.8–8.2 mg/100 g of iron in fava beans and 1.9–3.0 mg/100 g in peas. The authors also reported 4.3–5.0 and 3.3–6.2 mg/100 g of zinc in fava beans and peas respectively. The importance of iron in the diet has been widely reported and now has a multitude of health claims associated with it including contribution to normal cognitive function and metabolism, and reducing tiredness and fatigue (European Commission, 2016). Zinc has also been established to contribute to normal cognitive function as well as carbohydrate and fatty acid metabolism (European Commission, 2016). In the case of iron and zinc, pulse flours were between 131–283% and 142–168% higher than wheat flour respectively. Wheat flour can be considered a source of calcium, but is low in iron, zinc and potassium, all of which are higher in pulse flours.

Taking into account the recommended mineral intake laid out by European Food Safety Authority (2017) which is highlighted in Table 3, it is clear that the use of fava-bean and pea flours in food product development could make a substantial contribution to meeting these requirements through the diet.

3.4. Phytochemical composition

3.4.1. Total phenolic content and antioxidant activity

Total phenolic content (TPC) varied significantly across the different flour samples, from 68.27 mg GAE/100 g in wheat flour, to 387.52 mg GAE/100 g in fava-bean flour (Table 4. All pulse flours had significantly higher TPC compared to wheat flour ($p < 0.001$).Total phenols includes tannins and phenolic acids which can effect overall digestibility of pulse flour (Sarwar Gilani et al., 2012). Both green-pea and yellow-pea flour had significantly lower TPC than fava-bean flour which may result in greater protein bioavailability.

The highest antioxidant activity was also observed in fava-bean flour (250.81 mg AAE/100 g and 256.43 mg TE/100 g DPPH and FRAP activity respectively). Positive correlations were observed between TPC and antioxidant activity (DPPH $r^2 = 0.9898$; FRAP $r^2 = 0.9934$), indicating a significant relationship between phenolic content and antioxidant activity. These results are in agreement with those reported by Šibul et al. (2016a), who observed the highest antioxidant activity in fava-bean extracts compared with 6 other pulse vegetable extracts, including yellow-pea. Phenolic content and antioxidant activity of legumes vary significantly across the literature. The bioactivity of such extracts depends heavily on the extraction and assay conditions and the lack of standardisation in these methods can make comparisons difficult (Šibul et al., 2016b). From the results outlined in Table 4 however, it can be noted that the antioxidant activity increased by as much 567% (fava-bean flour) compared with wheat flour. The biological activities of these plant chemicals are continually becoming more understood and have the potential to play a preventative role in inflammatory conditions and metabolic syndrome, as highlighted in several reviews (Campos-Vega et al., 2010; Babu et al., 2013; Wang et al., 2014). The results presented in this study, demonstrates the potential of incorporating such flours into wheat based foods to increase the level of such beneficial plant chemicals in the diet. This was demonstrated by Turco et al. (2016) who used a wheat-pulse flour composite to increase the nutritive value of semolina pasta. They observed a significant increase of 190% in TPC and 18% in antioxidant activity (ORAC) of extracts from semolina pasta following the addition of fava-bean flour at 35%. Similarly, Millar et al. (2017) used wheat-pulse flour composites in the formulation of unleavened crackers. The authors reported an increase of up to 162% in TPC, and 182% in antioxidant activity (DPPH), following substitution of wheat flour for fava-bean, green- and yellow-pea flours at 35%.

Table 3
Mineral composition of fava-bean, green- and yellow-pea, and wheat flour (mg/100 g DM), requirements for making nutritional claims on foods, and recommended dietary intake of each mineral.

	Zinc	Magnesium	Calcium	Iron	Potassium
Fava bean	**4.18 ± 0.9^{a}**	*101.55 ± 3.89^{b}*	*172.65 ± 47.06^{a}*	**5.48 ± 0.84^{a}**	**1220.45 ± 53.83^{a}**
Green pea	**3.88 ± 1.28^{a}**	*103.2 ± 5.7^{ab}*	*113.53 ± 36.11^{a}*	*3.31 ± 0.36^{a}*	**1043.91 ± 17.14^{b}**
Yellow pea	**3.78 ± 1.13^{a}**	**114.2 ± 2.92^{a}**	*169.66 ± 27.48^{a}*	*3.92 ± 1.19^{a}*	**1099.16 ± 27.75^{b}**
Wheat	1.56 ± 0.42^{b}	*45.8 ± 4.64^{c}*	*174.66 ± 91.79^{a}*	1.43 ± 0.98^{b}	197.58 ± 6.12^{c}
High in	3.2	112.4	240	4.4	600
Source of	1.6	56.2	120	2.2	300
RI$^{\Delta}$	10	375	800	14	2000

Data presented as means ± standard deviation from triplicate analysis.
a–c Values followed by different superscripts in the same column are significantly different ($p < 0.05$).
Figures in bold are categorised as 'high in' the mineral (provide at least 30% of the RI in 100 g).
Figures in italics are categorised as 'source of' the mineral (provide at least 15% of the RI in 100 g) (Food Safety Authority of Ireland, 2016).
Δ Dietary reference value of minerals (mg/day) for adults (≥25yrs) (European Food Safety Authority, 2017).

Table 4
Phytochemical composition of fava-bean, green- and yellow-pea, and wheat flour.

	TPC mg GAE/100 g	DPPH mg AAE/100 g	FRAP mg TE/100 g	TIU/mg	PA mg/100 g	PA : Zinc$^{\Delta}$	PA : Iron
Fava bean	387.52 ± 14.32^{a}	250.81 ± 7.86^{a}	256.43 ± 30.37^{a}	5.45 ± 1.11^{a}	888.6 ± 37.58^{a}	1 : 213	1 : 162
Green pea	121.93 ± 1.00^{b}	57.66 ± 9.07^{b}	49.44 ± 12.70^{b}	3.69 ± 0.82^{b}	543.41 ± 25.62^{ab}	1 : 140	1 : 159
Yellow pea	129.85 ± 2.58^{b}	60.97 ± 10.56^{b}	58.98 ± 2.79^{b}	4.34 ± 0.38^{b}	574.14 ± 84.38^{a}	1 : 152	1 : 146
Wheat	68.27 ± 2.29^{c}	37.58 ± 11.73^{b}	19.76 ± 7.74^{c}	1.9 ± 0.30^{c}	356.66 ± 7.10^{b}	1 : 228	1 : 249

Data presented as means ± standard deviation from triplicate analysis.
a–c Values followed by different superscripts in the same column are significantly different ($p < 0.05$).
TPC: Total phenolic content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing anti-oxidant power; TIU: Trypsin inhibition units; PA: Phytic acid.
ΔRatio of mineral content (zinc and iron) to phytic acid content.

3.4.2. Phytic acid

Pulse flour had a higher content of phytic acid (PA) compared with wheat flour (Table 4). Fava-bean flour had the highest overall, 149% higher content than in wheat flour. This was higher than previously published values for chickpeas (600–790 mg/100 g) and lentils (675 mg/100 g) (Martín-Cabrejas et al., 2009; Pedrosa et al., 2012). It was however, lower than the value reported by Kumar et al. (2006) in soy (3170–3900 mg/100 g). Green and yellow-pea flour had a lower content of PA, 543 and 574 mg/100 g respectively. Wang et al. (2008) reported 640–830 mg/100 g PA across six different pea species, while Amarakoon et al. (2012) reported 490–710 mg/100 g PA in six species. While PA may have some beneficial health effects (Campos-Vega et al., 2010), high levels of consumption can be harmful to health due to their ability to form chemical complexes with minerals, particularly iron and zinc, reducing their absorption through the diet (Amarakoon et al., 2012). The mineral recommendations as set out by the European Food Safety Authority (2017) advise an increase in consumption of zinc in the case of high phytic acid intake (300–1200 mg/day) by 2–7 mg/day, to allow for sufficient zinc bioavailability. While wheat flour had the lowest PA content, it also had the lowest content iron and zinc. This resulted in a higher ratio of PA to both zinc and iron, compared with pulse flour (Table 4). A more favourable ration of PA to zinc and iron was observed in the pulse flours, in green pea flour which had the lowest ratio of PA to both zinc and iron. Amarakoon et al. (2012) also identified peas as a rich source of minerals with a naturally low content of phytic acid.

3.4.3. Trypsin inhibitory activity

Trypsin inhibitory activity was highest in the fava-bean flour and was significantly higher in all pulse flours compared with wheat flour, by 94–187%, $p < 0.01$, (Table 4). However, the pea flours had significantly lower TIU/mg than fava-bean flour, and these results were comparable to those by Pisulewska and Pisulewski (2000), who reported between 2.5 and 4.5 TIU/mg. The results observed in this study were lower than those reported by Guillamón et al. (2008) who observed between 5–10 and 6–15 TIU/mg, for both fava-bean and pea flours respectively. Lentils and chickpeas were reported to contain 4.9 and 11.7 TIU/mg respectively (Pedrosa et al., 2012), while soybeans can contain as much 55–63 TIU/mg (Pisulewska and Pisulewski, 2000). As with phytic acid, while they may have some health benefits, trypsin inhibitors have the potential to reduce digestibility and as such lower levels are desirable. From results presented in Table 4, pea flours have the more desirable TIU content compared with fava-bean flour.

4. Conclusions

Fava-bean flour had the highest protein content while green-pea flour had the highest dietary fibre content. All three pulse flours contained essential amino acids in adequate quantity, highlighting their potential application in developing protein-enriched foods. All pulse flours had significantly higher total mineral content compared with that of wheat flour, particularly for potassium and magnesium, with 100 g contributing to almost half of the recommended dietary intake. Fava-bean flour had significantly higher polyphenol content and antioxidant activity compared with green-pea and yellow-pea flours. However, green- and yellow-pea flours had a more favourable profile overall, considering their lower content of phytic acid and trypsin inhibitory activity, which would increase the bio-availability of proteins and minerals. The macro- and micro-nutrient profile of these pulse flours demonstrate their potential application in enhancing the nutritional quality of cereal based foods re-formulated with these flours. Further studies on the digestibility and bioavailability of these macro- and micro-nutrients will provide valuable information on the overall nutritional value of these foods to the consumer.

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Appendix A. Supplementary data

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