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The genotype and environment effects on the chemical composition and rheological properties of field peas.

Running title – "Genotype and environment effects on composition and rheology of field peas"

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Abstract

Background

The inclusion of pulses in traditional wheat-based food products such as bread, cakes and pasta are increasing as the food industry and consumers are recognising the nutritional benefits due to high protein, antioxidant activity and good source of dietary fibre of pulses. In all crops, including cereals, oilseeds and pulses, variability in chemical composition is known to exist due to genetic differences and environmental effects. This study reports the effect of genotype and environment on seed composition and rheological properties of field pea genotypes for both field pea flour and isolated starch.

Results

Genotype had significant effect on the chemical composition (protein, total starch, water soluble carbohydrates and phenolic compounds), the mean starch granule size and rheological properties (peak viscosity, breakdown viscosity, final viscosity, peak time & pasting temperature) of the field peas. Growing environment also had significant effect on starch granule size, phytic acid, water soluble carbohydrates, some phenolic compounds and pasting characteristics of field peas. G×E interaction were observed for protein, some phenolic compounds and some pasting characteristics.

Conclusion

Genotype and growing environment had a significant effect on the chemical composition and rheological properties of the field pea. The variability in composition and quality traits could be

advantageously exploited through plant breeding and optimised agronomic practices to increase production of field pea with desired quality traits.

Keywords: field peas, genotype, environment, composition, pasting characteristics

1. Introduction

Field pea (*Pisum sativum*) is a major pulse grown for human consumption as a source of protein, carbohydrates, minerals and bioactive phytochemicals contributing to improved metabolic health. In 2014 the worldwide production for field peas (dry) was 11.2 million tonnes.[1]

The major constituent of field pea is starch, which is comprised of two polymers of D-glucose; amylose and amylopectin. The arrangement of amylose, amylopectin and granule size has been reported to influence the pasting behaviour of starch including viscosity and pasting temperature which in turn affects its use as an ingredient in foods.[2,3] Due to the differences in physiochemical properties between pulse and cereal starches, starch from pulses can provide some unique characteristics to food systems including high gelation temperature, resistance to shear thinning, higher elasticity and high concentrations of resistant starch.[4] In addition field peas also contain bioactive compounds including oligosaccharides, phenolic compounds and phytic acid.[5]

Water soluble carbohydrate (WSC) of field pea consists of simple sugars and oligosaccharides. The raffinose family of oligosaccharides (RFOs) are of most interest in field pea studies. RFOs comprised of galactose molecules (linked by α-D-1, 6-glycosidic bonds) attached to sucrose.[6] Humans lack the necessary enzymes required to breakdown these RFOs and this leads to these oligosaccharides being digested by intestinal flora by way of anaerobic fermentation, leading to increased gas production in the intestinal tract.[7] A review by Rochfort[8], reported on the increased focus on reducing the amount of RFO found in legumes, due to their link with flatulence and other related disorders including, bloating and diarrhoea.

The major phenolic constituents found in field peas include condensed tannins, flavonoids and phenolic acids.[9] These phenolic compounds are found primarily in the seed hull and are biosynthesised through the phenylpropanoid pathway, with condensed tannin molecules being responsible for the seed-coat colour.[10] In dark-coloured hulls, tannin and flavonoid compounds make up the large majority of the phenolic compounds, while in seeds with clear hulls, phenolic acids represent the major compounds.[11] Phenolic compounds in the seed coat, exhibit antioxidant and anti-mutagenic activity protecting the seed from oxidative damage.[12] In the field, these compounds are also thought to provide a chemical defence during the growing stage against pathogens and insect pests.[13] Phenolic acids in field peas occur primarily as the insoluble or bound forms, covalently bonded to structural components of the cell wall such as cellulose, hemicellulose, lignin and pectin.[14,15] The phenolic composition of field peas has become of particular interest associated with metabolic health given their reputed protective properties against oxidative damage.[9,16] In reviews by Campos-Vega[5] and Rochfort[8], isoflavone phenolic compounds have been associated with biological activities in the reduction of osteoporosis, cardiovascular disease, prevention of cancer and treating symptoms of menopause. Phenolic compounds also exhibit anti-nutritional effects and studies have shown reduction in the bioavailability of proteins caused by phenolic compounds.[17]

Phytic acid functions as a storage for phosphate and minerals in seeds which can be retrieved during germination.[18] Phytic acid has been identified as an anti-nutrient due to its ability to chelate with multivalent ions especially Zn, Ca and Fe, inhibiting the ability for the body to uptake dietary minerals by reducing their bioavailability.[17]

There is an increasing interest in utilising pulses in wheat-based products with blends.[19] The demand for gluten-free baked products has led to exploration of nutritional properties of baked good from pulses like chickpea and lupins.[20] The exploitation in rheological properties of field pea flour including gelation properties of starch would be advantageous in examining the potential application of filed pea flour in baked products. The new areas of pulse usage could increase the demand for pulses with specific nutritional and rheological characteristics which in turn increase the need for exploration of factors affecting nutritional and functional properties of pulses.

The aim of this study is to investigate the effects of genotype, environment and G × E interaction on the range of compositional and rheological parameters of field pea grains.

2. Materials and Method

2.1. Seed material

Nine field pea genotypes (4 Kaspa-type, 4 Dun-type and 1 White; Table 1) were obtained from two diverse field trial sites in Australia. The two growing sites were Sea Lake (35.50°S, 142.85°E) with calcareous loam soil type and Balaklava (34.15°S, 138.42°E) with sandy loam/medium clay soil type. The monthly rainfall and monthly mean maximum temperature for each trial sites are as shown in Table 2. Each genotype was replicated 3 times at each trial site.

2.2. Sample Preparation

The extraction of phenolic compounds from pulse samples was adapted from Luthria [21]. Field pea samples were dehulled using an abrasive stone disk dehuller, the hulls were separated from

the cotyledon. The cotyledon and hull samples were ground using a Perten Falling Number hammer mill 3100 fitted with a 0.8 mm screen (Perten Instruments, Hägersten, Sweden).

2.3. Compositional analysis

2.3.1. Protein Analysis

Percent protein was determined by analysing 0.2 g flour samples using a LECO TruMac Nitrogen Analyser (LECO Corporation, Saint Joseph, USA) and reported on an 'as is' basis.

2.3.2. Phytic Acid Assay

Phytic acid content of field pea cotyledon was determined using the Phytic acid (phytate)/ total phosphorus assay kit (K-PHYT; Megazyme International, Wicklow, Ireland).

2.3.3. Total Starch

Total starch content of the samples was analysed using Megazyme Total Starch assay kit (K-TSTA 07/11; Megazyme International, Wicklow, Ireland).

2.3.4. Starch Extraction

Starch was extracted using a modified method adapted from Hood-Niefer[2]. 40 g of cotyledon flour and deionised water were added to a beaker at a ratio of 1:3 (w/v) and stirred for 15 minutes and sieved through 250 μm and 75 μm sieves. The homogenate was then collected and resuspend in deionised water and stirred for 15 minutes before being sieved. The starch suspensions were combined and centrifuged at 10000 ×g (Sorval RC 6+, Thermo Fisher Scientific, Massachusetts, USA) for 10 minutes. The supernatant was then discarded, and the top gelatinous layer was carefully removed from the hard starch pellet. The starch pellet was

resuspended in 100 mL of deionised water and centrifuged again for 10 minutes at 10000 ×g. Again the supernatant was discarded, and the protein layer removed. This procedure was repeated three times. The pellet was resuspended in 100 mL of 0.05 M NaOH and stirred for 1 hour. The 0.05 M NaOH was neutralised using 0.1 M HCl and the pH was adjusted to 7.0. The suspension was filtered through Whatman No. 3 filter paper using a vacuum pump. The starch pellet was washed in 100 mL of ethanol and then rinsed with acetone. The starch pellet was dried overnight and stored at -20°C until further analysis.

2.3.5. Amylose

The amylose assay method was adapted from Kaufman [22]. To 5 mg of starch weighed in 2 mL Eppendorf tubes, 1 mL of DMSO solution (9:1 mixture of dimethyl sulfoxide : deionised water) was added. The mixture was mixed on Eppendorf Thermo-Shaker for 1 h at 95°C and 1000 rpm. The 100 μL amylose calibration standards were prepared with following proportion of amylose : amylopectin samples (100 μL : 0 μL, 80 μL : 20 μL, 60 μL : 40 μL, 40 μL : 60 μL, 20 μL : 80 μL and 0 μL : 100 μL) into 2 mL Eppendorf tubes. Also pipette 100 μL of sample extract into 2 mL Eppendorf tubes. Then add 100 μL of 3.04 g/L iodine in DMSO solution into each tubes and vortex the mixture. Pipette 20 μL of each reaction mixture into 96-well plate. Add 180 μL of deionised water to each well, mix for 1 min and read absorbance at 620 nm and 510 nm.

2.4. Particle size analysis

0.5 g of dry starch was suspended in 5 mL of deionised water and stirred for 15 minutes. Samples were analysed through a Malvern Mastersizer Hydro 2000MU (Malvern Panalytical Ltd, Malvern, United Kingdom)

2.5. Water Soluble Carbohydrate (WSC)

2.5.1. WSC Extraction

0.2 g of ground field pea cotyledon and 5 mL of deionised water were briefly vortexed to suspend the ground cotyledon. The samples were sonicated for 10 minutes. The vortex and sonication procedure were repeated twice and then centrifuged at 3220 ×g (Eppendorf Centrifuge 5810, Hamburg, Germany) for 10 minutes. 0.75 mL of the supernatant was transfer to a 2.0 mL Eppendorf tube and was diluted 1:1 with acetonitrile. The samples were then centrifuged at 10600 ×g (Eppendorf Centrifuge 5430R, Hamburg, Germany) for 10 minutes. The supernatant was then filtered through a 0.22 μm syringe filters.

2.5.2. Water Soluble Carbohydrate UPLC Analysis

Analysis of WSC was performed using a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) equipped with UPLC Binary Solvent Manager, UPLC Sample Manager and an Evaporative Light Scattering Detector (ELSD). Separation was performed using a Waters ACQUITY BEH Amide column (2.1×100 mm, 1.7 μm) at 25°C. The mobile phase consisted of 80% acetonitrile with 0.05% ammonia (Solvent A) and 30% acetonitrile with 0.05% ammonia (Solvent B). The injection volume was 2.0 μL for all samples and the flow rate was kept constant at 0.13 mL/min over a 20:30 minute run time. UPLC ELSD data were analysed using Empower 3.

2.6. Phenolic extractions and quantification

Ground field pea hull (0.25 g) of samples were weighed into 50 mL tubes to which 7.5 mL of 13.4 g/L ascorbic acid in 13.4 mM of EDTA solution was added and then vortexed. 2.5 mL of 8 M NaOH was then added and the samples were vortexed and incubated in 50°C water bath for 30 minutes. The samples were vortexed every 5 minutes during this incubation period. The samples were then acidified with the addition of 1.65 mL of 10.2 M HCl and incubated at 50°C water bath for 30 minutes. 12.5 mL of ethyl acetate was added to each sample and mixed in an end-over-end rotator for 5 minutes. Samples were centrifuged at 3220 ×g for 10 minutes. The organic phase was then pipetted into a 50 mL centrifuge tube. This separation process was repeated and both organic phases were combined and evaporated to dryness at 60°C using a dry block heater under a stream of nitrogen gas. The dried residue was dissolved in 2 mL of methanol solution (1:1 mixture of methanol : water). The redissolved residues were then filtered using syringe filters.

The phenolic compounds were quantified using a Waters UPLC system equipped with an ACQUITY photodiode array (PDA) detector over a range of 210 nm to 400 nm for 3D analysis and 280 nm for 2D analysis using Empower software 3. Separation was performed using UPLC BEH C18 column (2.1 x 50 mm, 1.8 μm) at 45°C. The mobile phase consisted of acetonitrile with 0.1% acetic acid (Solvent A) and MilliQ water with 0.1% acetic acid (Solvent B). The injection volume for each sample was 2.0 μL and the flow rate was kept constant at 0.8 mL/min over a total run time of 8 minutes. The solvent gradients were run as followed (mm:ss): 00:00-0:30 minutes: isocratic flow of 1% Solvent A and 99% solvent B, 00:30-04:00 minutes: linear gradient to 30% solvent A and 70% solvent B, 04:00-05:30 minutes: linear gradient to 95%

solvent A and 5% solvent B, 05:30-06:30 minutes: linear gradient to 1% solvent A and 99% solvent B, 06:30-08:00 minutes: isocratic flow of 1% solvent A and 99% solvent B.

The peaks were validated using mass spectroscopy using Waters QDa mass detector. The settings for QDa were as follow: Capillary voltage: 0.8 kV; Polarity: negative; Cone voltage: 10 V; Probe temperature: 600°C; Scan range: 100–1200 m/z and Data rate: 5 Hz.

2.7. RVA Analysis

Peak viscosity and pasting temperature for pulse flour samples (12.6% db total weight 28.5 g) were determined by using a Rapid Visco Analyser RVA-4 (Perten Instruments, Hägersten, Sweden). The RVA profile was run as follows (mm:ss): 0:00 - 0:00 50°C 960 rpm, 0:00 – 0:10 50°C 160 rpm, 0:10 – 1:00 50°C 160 rpm, 1:00 – 4:42 95°C 160 rpm, 4:42 – 7:12 95°C 160 rpm, 7:12 – 11:00 50°C 160 rpm, 11:00 -13:00 50°C 160 rpm, 13:00 End.

2.8. Statistical Analysis

Statistical analyses were carried out on 3 replicates to compare nine field pea genotypes at two locations. All analyses were conducted using GenStat® (18th Edition; VSNI, Hempstead, UK). The data, from the experimental design were analysed for the multiple experiments via a meta-analysis using the method of residual maximum likelihood (REML).

3. Results and Discussion

3.1. Compositional analysis

Seed protein varied significantly between genotypes (p<0.001) and ranged from 235 g/kg (OZP1401) to 261 g/kg (PBA Oura) for Balaklava and 234 g/kg (OZP1405 & Sturt) to 261 g/kg (PBA Oura) for Sea Lake which are comparable to field pea protein values reported by others[2]. However, no significant differences in protein content was observed between environments (Table 3). A significant G×E effect was noted on the seed protein contents (p<0.05). Compared to cereal grains, field pea contains much higher level of proteins. Hence, inclusion of field peas in the production of traditionally cereal based products (e.g. bread, pasta and biscuits) will lift the protein content of the products.[19]

Phytic acid in grains is of interest as its presence is known to affect the nutritional value of grain as when consumed by humans and animals it binds with essential minerals (calcium, iron, zinc etc) thereby reducing their bioavailability.[23] Environment had a significant effect on phytic acid concentration (P<0.05; Table 3). The values ranged from 6.3 g/kg to 8.1 g/kg at Balaklava and 4.8 g/kg to 7.5 g/kg at Sea Lake. These values are in accordance with other studies on phytic acid concentrations in field peas.[24,25] Previous studies linked the concentration of phytic acid with growing environment, the highest phytic acid concentration being reported from genotypes grown in soils rich in phosphorous.[26] However, significant difference in the phytic acid concentration between genotypes was not observed. The ash content of samples ranged from 23.9 g/kg to 27.7 g/kg (Table 3). There was no significant effect of genotype, environment and G×E interactions on the ash content of pea flour. However, the ash content was strongly correlated with phytic acid contents (r=0.79). The most probable reason for this strong correlation is that phytic acid strongly bind metal ions.[27]

Starch has important visco-rheological properties in food and therefore its concentration and composition is of interest.[28] The total starch content of field peas varied between genotypes (p<0.001; Table 3) and ranged from 386 g/kg (Kaspa) to 430 g/kg (OZP1201). There was no significant difference in starch concentration between environments and similarly no interactive effects were observed. Amylose forms part of dietary fibre as these crystalline starch molecules are resistant to digestive enzymes of humans. The amylose content of field pea starch ranged from 398 to 450 g/kg of starch, but there was no significant difference between genotype and environment. Hood-Niefer [2] also did not find any significant difference in the amylose contents of 10 pea genotypes grown in four different locations in Canada.

The median starch granule size ranged from 22.5 μm (OZP1401; Balaklava) to 27.6 μm (OZP1201; Sea Lake) (Table 4), which are similar to the reported mean particle size of peas starches [3,29]. There was a significant effect of genotype (p< 0.001) and environment (p<0.05) on the median starch granule size. Kaspa had comparatively smaller starch granules whereas Sturt had larger starch granules (Figure 1). On average, the samples from Sea Lake had slightly larger starch granules. There was no G × E effect on the mean starch granule size.

3.2. Water soluble carbohydrates

Glucose, sucrose, maltose, raffinose, stachyose and verbacose were identified in all nine genotypes (Table 5). The genotype had very significant effect on the WSC profile of field peas (p<0.001), for all sugars and RFOs except for maltose (p<0.05). The environment had significant effect on all WSC parameters (p<0.05) except for verbacose. On average the field

peas from Balaklava site had 3.3 g/kg of additional total WSC compared to genotypes grown at Sea Lake, 98.3 g/kg compared to 95.0 g/kg of total WSC.

Stachyose, verbacose and sucrose was found to comprise most of WSC observed in the cotyledon (Table 5). Stachyose concentration ranged from 26.9 g/kg to 39.1 g/kg, verbacose ranged from 13.7 g/kg to 26.6 g/kg and sucrose ranged from 20.7 g/kg to 26.6 g/kg. Raffinose was the next most abundant WSC ranging from 9.4 g/kg to 13.0 g/kg. These results are in line with a similar study previously reported.[25]

The raffinose family of oligosaccharides have been reported as the major soluble carbohydrates in field peas. On one hand they are linked with causing flatulence and other bowel discomforts in humans.[24,25] On the other hand they are promoted as prebiotics for improved digestive system.[30] Suarez [31] stated that consumption of product having less than 3.1 g of RFOs did not significantly increase flatulence frequency. Several methods have been reported to reduce the concentration of RFOs in pulses, including soaking, coking, germinating or fermenting the seeds.[32] As genotype and growing environment had significant effect on the RFOs compositions (Table 5), pulse breeding and agronomic practise could be a potential tool to produce pulses with optimum level of RFOs.

3.3. Phenolic acids and catechins

The phenolic acids in the seed coat have been reported as either free phenolic acids or phenolic acid derivatives. Significant differences between genotype in the concentration of catechin (p<0.001) and all phenolic acids (3,4-dihydroxybenozic, *p*-coumaric, *trans*-ferulic, p-coumaroyl

malic and *trans*-feruloyl malic) (p<0.001; Table 6) were observed. The most abundant compound 3,4-Dihydroxybenzoic acid ranged in concentration from 32 mg/kg in Sturt to 217 mg/kg in OZP1405 at Balaklava. Similar concentration range of 3,4-dihydroxybenzoic acid were found in samples at Sea Lake. Differences due to environment was only observed to have a significant effect on concentrations of two most abundant free phenolic acids 3,4-dihydroxybenzoic acid (p<0.001) and *trans*-Feruloyl malic acid (P<0.01). The genotype by environment interactions where observed only for 3,4-dihydroxybenzoic acid (p<0.001) and *p*-coumaroyl malic acid (p<0.05). Dueñas[12] reported 1.48 mg/kg and 4.49 mg/kg of *p*-coumaric acid and *trans*-ferulic acid respectively in the seed coat, nevertheless this study reports higher amounts of these phenolic acids, 8.9 to 16.2 mg/kg *p*-coumaric and 14.4 to 24.5 mg/kg *trans*-ferulic acid. These differences are most likely attributed to Dueñas [12] not performing an acid or base hydrolysis during anlayses to liberate the bound phenolic acids, which exist in insoluble bound complexes bound through ester and glycosidic linkages to cell wall polymers.[33]

Catechin concentration ranged from 12 mg/kg (Sturt) to 2303 mg/kg (PBA Gunyah) at Balaklava and 32 mg/kg (Sturt) to 2291 mg/kg (PBA Twilight) at Sea Lake (Table 6). From the genotypes analysed in this study only Sturt has a clear-coloured hull and had least amount of catechins and total phenolic acids. On the contrary, Kaspa-type field peas with tan-coloured hulls had the most abundant amount of catechins and total phenolic acids (Table 6). These results indicate that the phenolic compounds in hulls are associated with seed-coat colour and genotype is the main governing factor for the concentration of phenolic compounds.

Due to the presence of phenolic compounds with an array of health benefits [8], the field pea hulls potentially be used as a valuable food ingredients to enhance the nutritional profile of food products which in turn would expand the crop utilisation.

3.4. Pasting characteristics

There has been increased interest in incorporating pulse flour to make wheat-based products such as bread with lentil flour blends[19,34]; baked crackers with pulse flours blends[35] and legume cakes[36]. The inclusion of pulse flour as blends with wheat flour would most likely bring along changes in the dough and starch rheology. The final viscosity, peak time and peak temperature could affect the processing conditions. In fact, Singh[37] has demonstrated that peak, trough and final viscosity were strongly correlated with hydration capacity of seeds. The genotype had a significant effect on all RVA pasting characteristics ($p<0.05$) except for breakdown viscosity for Sea Lake. The peak viscosity ranged from 1308 cP (Kaspa) to 1819 cP (OZP1201), breakdown viscosity ranged from 16 cP (OZP1405) to 209 cP (OZP1401) and final viscosity ranged from 2085 cP (Sturt) to 2948 cP (OZP1201). Similarly, the peak time ranged from 5.1 min (Sturt) to 6.8 min (OZP1405) and pasting temperature ranged from 77.1°C (OZP1401 and PBA Oura) to 80.8°C (Kaspa). Overall, the field pea genotypes with comparatively bigger starch granules (e.g. Sturt) had comparatively higher viscosities and lower temperatures in their pasting characteristics. These finding are similar to that of Shen[38] who showed that high protein pea genotype, which had on average smaller starch granules compared to other 5 pea types, had the highest gelatinisation temperature. The environment also had a significant effect of all starch-pasting characteristics ($p<0.05$; Table 7). The mean peak viscosity, breakdown viscosity and final

viscosity were higher for samples from Sea Lake compared to samples from Balaklava. However, the peak time and pasting temperature were comparatively lower for samples from Sea Lake. The most probable reason for these observations is the differences in starch granules, the mean starch granule size for samples from Sea Lake were slightly bigger compared to samples from Balaklava. The largest change due to trial site was observed for breakdown viscosity, 96% increased from Balaklava (73 cP) to Sea Lake (143 cP). The mean pasting temperature for the samples from Balaklava were on average 1.9°C higher compared to samples from Sea Lake.

There were significant G × E interactions for peak viscosity, trough viscosity, final viscosity, setback viscosity and peak time ($p<0.05$). No G × E interactions were observed for breakdown viscosity and pasting temperature.

The median starch granule size was negatively correlated ($p<0.05$) with peak time ($r = - 0.47$) and pasting temperature ($r = - 0.54$); and it was positively correlated ($p<0.05$) with peak viscosity ($r = 0.35$) and breakdown viscosity ($r = 0.27$). The peak time and pasting temperature were positively correlated ($p<0.05$) to ash content, phytic acid, total WSC content, total phenolic acids and catechin contents (r ranging from 0.33 to 0.55). This could be due to the solute effect on the pasting temperature i.e. increasing the solute concentration increases the gelatinisation temperatures of starches.[39]

4. Conclusion

Genotype had significant effect on the chemical composition (protein, total starch, WSC and phenolic compounds) and rheological properties of the field pea. Growing environment also had significant effect on the starch granule size, WSC profile, some phenolic compounds and pasting

characteristics of field pea. Hence, pulse breeding and agronomic practice would be a great tool in production of field pea with desired quality traits.

Acknowledgments

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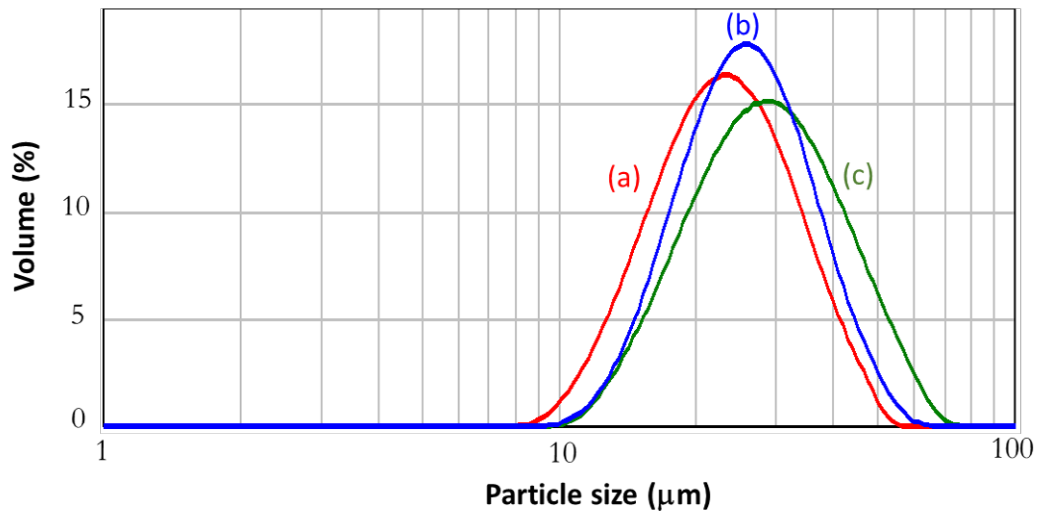
Figure 1: Example of particle size distribution of starches obtained using Mastersizer 2000 particle size analyser for field pea samples [(a) Kaspa (b) PBA Oura and (c) Sturt] grown in Sea Lake

Table 1: Seed characteristics of nine field pea genotypes

Genotype	Market grade	Seed shape	Seed colour	
			Hull	**Cotyledon**
Sturt	White/Yellow *	Smooth round	Clear	Yellow
Kaspa			Tan	Yellow
OZP1405	Kaspa type	Smooth round, minimal dimpling	Tan & mottled	Yellow
PBA Gunyah			Tan	Yellow
PBA Twilight			Tan	Yellow
OZP1201			Green & Tan	Yellow
OZP1306	Dun type	Dimpled (flattened face)	Tan	Yellow
OZP1401			Tan	Yellow
PBA Oura			Green & Tan	Yellow

* White in Australia & yellow in Canada/North America

Table 2: Monthly rainfall and monthly mean temperature during field pea growing season in 2014 at two trial sites.

	Trial site	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Monthly total rainfall - mm	Balaklava	48.8	103.3	32.4	0.0	21.9	3.6	3.0	4.6
	Sea Lake	24.2	23.2	18.2	2.8	55.6	5.8	22.6	7.8
Monthly mean maximum temperature - °C	Balaklava	18.1	13.5	12.8	14.7	18.6	25.7	27.0	27.1
	Sea Lake	20.2	15.6	15.0	17.7	21.7	27.5	29.1	30.5

Meteorological data were obtained from The Bureau of Meteorology

(http://www.bom.gov.au/climate/data/)

Table 3: Composition of field pea cotyledon (g/kg), for nine field pea genotypes grown at two different sites (Balaklava and Sea Lake).

Market grade	Genotype	Protein		Starch				Ash		Phytic Acid	
				Total starch (in cotyledon)		Amylose (in starch)					
		Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake
Kaspa type	Kaspa	243	256	391	386	398	435	26.9	27.2	7.3	7.5
	OZP1405	240	234	420	415	425	424	25.7	23.9	7.0	4.8
	PBA Gunyah	243	240	413	412	434	422	25.9	27.7	7.0	7.4
	PBA Twilight	241	242	411	406	427	430	26.8	26.1	6.7	6.1
Dun type	OZP1201	255	252	423	430	427	412	26.2	24.3	8.1	6.2
	OZP1306	240	241	409	415	411	411	27.0	25.6	7.3	5.5
	OZP1401	235	248	424	419	424	450	24.7	24.7	6.3	6.3
	PBA Oura	261	261	429	414	425	424	25.3	24.9	7.2	5.6
White	Sturt	243	234	415	420	447	422	26.5	25.5	6.7	5.8
	Grand Mean	245	245	415	413	424	426	26.1	25.5	7.0	6.0
Statistical significance	Genotype (location specific)	***	**	*	**	NS	NS	*	NS	NS	NS
	Genotype (pooled)	***		***		NS		NS		NS	
	Environment	NS		NS		NS		NS		*	
	G × E	*		NS		NS		NS		NS	
L.S.D of means	Genotype	7		13		20		1.5		1.6	
	Environment	3		6		10		0.7		0.8	
	G × E	10		18		29		2.1		2.3	

*Significance: *P < 0.05, **P <0.01, ***P <0.001. NS: Not Significant*

Table 4: Median starch granule size of field pea starch, for nine field pea genotypes grown at two different sites (Balaklava and Sea Lake).

Market grade	Genotype	Median starch granule size (µm)	
		Balaklava	Sea Lake
Kaspa type	Kaspa	23.1	23.5
	OZP1405	23.2	23.2
	PBA Gunyah	25.0	26.1
	PBA Twilight	23.6	24.1
Dun type	OZP1201	25.7	27.6
	OZP1306	23.5	24.3
	OZP1401	22.5	24.1
	PBA Oura	24.8	25.6
White	Sturt	27.1	27.1
Grand Mean		**24.3**	**25.1**
Statistical significance	Genotype (location specific)	*	***
	Genotype (pooled)	***	
	Environment	*	
	G × E	NS	
L.S.D of means	Genotype	1.2	
	Environment	0.6	
	G × E	1.7	

Significance: *P < 0.05, **P <0.01, ***P <0.001. NS: Not Significant

Table 5: Water soluble carbohydrates (g/kg) cotyledon, for nine genotypes grown at two trial sites, Balaklava and Sea Lake.

Market grade	Genotype	Glucose		Sucrose		Maltose		Raffinose		Stachyose		Verbacose	
		Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake
Kaspa type	Kaspa	2.3	2.6	26.6	25.7	6.0	5.0	12.0	9.8	34.3	26.9	19.7	23.7
	OZP1405	2.2	2.6	21.9	24.6	5.8	4.2	10.8	9.9	34.4	31.4	25.6	23.6
	PBA Gunyah	2.2	2.7	25.3	26.0	5.4	4.1	11.5	10.4	34.0	29.8	23.4	24.2
	PBA Twilight	2.1	2.4	25.0	26.0	4.6	4.0	10.3	10.1	32.4	31.2	24.7	24.9
Dun type	OZP1201	2.7	3.1	20.7	23.5	5.6	4.1	10.3	9.8	31.8	28.1	25.0	25.9
	OZP1306	2.0	2.4	22.4	24.0	4.7	4.2	10.9	10.0	34.0	32.2	23.7	24.2
	OZP1401	2.0	1.9	24.8	26.3	3.6	3.2	9.6	9.4	29.4	28.0	24.0	23.0
	PBA Oura	2.3	3.5	20.7	21.4	5.0	4.4	13.0	11.5	39.1	34.2	13.7	15.4
White	Sturt	2.1	2.7	26.2	26.1	5.3	4.1	10.4	9.7	31.3	27.3	26.6	26.1
Grand Mean		**2.2**	**2.7**	**23.7**	**24.8**	**5.1**	**4.1**	**11.0**	**10.1**	**33.4**	**29.9**	**22.9**	**23.4**
Statistical significance	Genotype (location specific)	NS	**	**	NS	*	NS	**	**	*	***	***	***
	Genotype (pooled)	***		***		*		***		***		***	
	Environment	***		*		***		***		***		NS	
	G × E	NS		NS		NS		NS		NS		NS	
L.S.D. of means (g/kg)	Genotype	0.4		2.2		0.9		0.8		2.3		2.0	
	Environment	0.2		1.0		0.4		0.4		1.1		1.0	
	G × E	0.6		3.1		1.3		1.1		3.2		2.9	

*Significance: *P < 0.05, **P <0.01, ***P <0.001. NS: Not Significant.*

Table 6: Mean values of flavanols and phenolic acids (mg/kg) in seed coat for nine genotypes grown at two different sites

Market grade	Genotype	Flavonol		Phenolic acids and its derivatives(mg/kg)											
		Catechin (mg/kg)		3, 4 Dihydroxybenzoic Acid		*p*-Coumaric Acid		*p*-Coumaroyl malic acid		*trans*-Ferulic Acid		*trans*-Feruloyl malic acid		*Total phenolic acid*	
		Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake
Kaspa type	Kaspa	2193	1563	203	178	11.9	10.8	61	47	17.9	17.2	63	36	357	289
	OZP1405	1729	1507	217	249	10.3	10.6	38	32	19.3	20.2	78	47	363	359
	PBA Gunyah	2303	2024	216	237	11.8	11.4	82	80	14.4	15.3	51	52	375	396
	PBA Twilight	2238	2291	193	220	11.6	10.8	78	78	17.4	17.9	59	62	359	389
Dun type	OZP1201	431	418	158	134	16.2	14.0	78	62	23.9	22.0	104	95	380	327
	OZP1306	1717	1435	141	208	9.9	9.0	40	57	15.2	15.4	9	9	215	298
	OZP1401	93	157	68	87	9.3	8.9	51	38	24.3	24.0	46	36	199	194
	PBA Oura	105	151	53	73	10.0	11.5	79	90	22.6	22.8	30	30	195	227
White	Sturt	12	32	32	34	12.6	13.9	48	32	20.9	24.5	66	41	180	145
	Grand Mean	1202	1064	142	158	11.5	11.2	62	57	19.5	19.9	56	45	291	292
Statistical significance	Genotype (location specific)	***	***	***	***	***	***	***	***	***	***	***	***	***	***
	Genotype (pooled)	***		***		***		***		***		***		***	
	Environment	NS		***		NS		NS		NS		**		NS	
	G × E	NS		***		NS		*		NS		NS		**	
L.S.D. of means	Genotype	295		18		1.1		10		1.5		16		33	
	Environment	139		8		0.5		5		0.7		8		16	
	G × E	416		25		1.6		14		2.1		23		47	

Significance: *P < 0.05, **P <0.01, ***P <0.001. NS: Not Significant.

Table 7: Pasting characteristics of field pea cotyledon flour

Market grade	Genotype	Peak Viscosity (cP)		Breakdown viscosity (cP)		Final viscosity (cP)		Peak time (min)		Pasting temperature (°C)	
		Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake	Balaklava	Sea Lake

Kaspa type	Kaspa	1308	1417	19	107	2241	2306	6.2	5.6	80.8	78.7
	OZP1405	1372	1607	16	142	2360	2638	6.8	5.7	79.8	78.7
	PBA Gunyah	1521	1671	70	96	2558	2911	5.9	5.6	80.3	78.2
	PBA Twilight	1399	1642	41	144	2362	2692	6.0	5.5	80.3	78.1
Dun type	OZP1201	1611	1819	110	173	2589	2948	5.7	5.4	80.5	77.3
	OZP1306	1383	1697	19	110	2333	2885	6.5	5.7	80.3	79.2
	OZP1401	1642	1818	103	209	2796	2892	5.7	5.3	79.2	77.1
	PBA Oura	1785	1550	160	171	2931	2275	5.6	5.2	79.2	77.1
White	Sturt	1649	1462	117	133	2494	2085	5.4	5.1	77.9	76.3
Grand Mean		1519	1632	73	143	2518	2626	6.0	5.5	79.8	77.9
Statistical significance	Genotype (location specific)	***	***	***	NS	*	***	***	*	***	***
	Genotype	***		***		***		***		***	
	Environment	***		***		*		***		***	
	G × E	***		NS		***		*		NS	
L.S.D. of means	Genotype	95		47		215		0.3		0.7	
	Environment	45		22		101		0.1		0.3	
	G × E	135		66		304		0.4		1.0	

Significance: *P < 0.05, **P <0.01, ***P <0.001. NS: Not Significant.

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