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Dough properties and baking characteristics of white bread, as affected by addition of raw, germinated and toasted pea flour

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ABSTRACT

Thermal and non-thermal processing may alter the structure and improve the techno-functional properties of pulses and pulse flours, increasing their range of applications in protein-enhanced foods. The effects of germination and toasting of yellow peas (*Pisum sativum*) on flour and dough characteristics were investigated. Wheat flour was substituted with raw, germinated and toasted pea flour (30%). The resulting bread-baking properties were assessed. Toasting increased dough water absorption and improved dough stability compared with germinated and raw pea flour ($p < 0.05$). This resulted in bread loaves with comparable specific volume and loaf density to that of a wheat flour control. Significant correlations between dough rheological properties and loaf characteristics were observed. Addition of pea flours increased the protein content of the breads from 8.4% in the control white bread, to 10.1–10.8% ($p < 0.001$). Toasting demonstrated the potential to improve the techno-functional properties of pea flour. Results highlight the potential application of pea flour in bread-making to increase the protein content.

1. Introduction

Legume seeds, or pulses (beans, peas, chickpeas and lentils) have grown in popularity as a source of plant protein, particularly since they were celebrated in 2016 with the International Year of the Pulse. As well as being an important source of protein, pulses are rich in carbohydrates and dietary fibre and have long been associated with good nutrition and linked to a range of health benefits (Abeysekara, Chilibeck, Vatanparast, & Zello, 2012; Messina, 2014; Ramdath, Renwick, & Duncan, 2016). While pulse grains are a staple in Eastern diets, consumption in Western countries remains low and they continue to be harvested largely for animal feed (Foyer et al., 2016). Consumption usually takes the form of cooked meal or as dry grains, following roasting/toasting. When milled into flour, pulses can be a valuable ingredient for food fortification. Their high lysine, low methionine profile offers a complementary source of protein to wheat flour (Masey O'Neill et al., 2012). Fortification of cereal-based foods with pulse flours can improve the protein content and compensate for the lysine and threonine deficiencies of wheat flour (Rutherfurd, Bains, & Moughan, 2012).

Bread is a universally consumed product and its versatility means it continues to evolve to reflect consumer needs, as well as advancements in ingredients, equipment and materials. While bread consumption has been undergoing a gradual decline in recent years, it still continues to make a substantial contribution to the diets of many cultures (Cauvain, 2015). Substitution of wheat flour with nutrient-rich pulse flours offers a viable method for increasing protein in the diet, particularly in diets where cereals make up a large part of the caloric intake (Bar-El Dadon, Abbo, & Reifen, 2017).

The structure, rheology and quality of bread are highly influenced by the starch-protein complex, and in particular, the presence of gluten. The use of non-wheat flour can interfere with the gluten network, resulting in a weakened bread dough and deterioration in bread quality (Collar, Jiménez, Conte, & Fadda, 2014). As a result of these constraints, there has been limited success in supplementing breads with a substantial amount (> 15%) of pulse flours without significant negative effects on the technological properties and bread quality (Mohammed, Ahmed, & Senge, 2014; Mondor, Guévremont, & Villeneuve, 2014; Sadowska, Błaszczak, Fornal, Vidal-Valverde, & Frias, 2003). The use of raw pulse flours has also been limited by the presence of anti-nutritional compounds which can reduce mineral absorption and protein digestibility, and non-digestible oligosaccharides which result in gastrointestinal problems (Campos-Vega, Loarca-Piña, & Oomah, 2010;

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Roopashri & Varadaraj, 2014).

Processing methods, such as thermal treatments or germination have been shown to remove or significantly reduce these compounds (Marchais, Foisy, Mercier, Villeneuve, & Mondor, 2011; Roopashri & Varadaraj, 2014). Such methods are also believed to positively affect the functional properties of these flours by altering the chemical composition. Germination involves sprouting of the seedling from the plant seed and has been used as a cost effective method for improving the nutritional profile and functional properties of both cereal and legume seeds (Dueñas et al., 2016; Elkhalifa & Bernhardt, 2010). For germination of pulses, which are the mature seeds from legumes, the seeds are rehydrated by steeping in water which allows the resumption of cellular metabolism and growth. This results in the activation of hydrolytic and proteolytic enzymes which break down macronutrients, releasing beneficial nutritional compounds and modifying the functional properties of the starch and protein fractions, thereby improving digestibility (Dueñas et al., 2016). Thermal treatments such as roasting/toasting pulse seeds are also reported to improve nutrient digestibility by the destruction or inactivation of certain heat labile anti-nutritional compounds such as low molecular weight proteins which can de-activate digestive enzymes (Ouazib, Garzon, Zaidi, & Rosell, 2016). Changes in protein and starch structures can also occur following germination or thermal processing, which may improve the emulsifying and foaming activities of pea flour (Benítez et al., 2013; Ouazib et al., 2016).

While studies have reported on the effects of these methods on the nutritional and functional properties of some pulse flours (Benítez et al., 2013; Dueñas et al., 2016), there is scarce information on using the resulting flours in bread-making. The objective of this research was to substitute wheat flour by 30% with high-protein yellow pea flour while maintaining the consistent quality of white bread. In particular, the effects of germination and toasting of yellow peas on the structural properties of the grains and milled flour blends, the dough rheology and baking characteristics of the resulting breads were assessed.

2. Materials and methods

2.1. Materials

Commercial wheat flour (Shackelton's Milling, Co. Meath, Ireland), split yellow peas (Hodmedod Ltd., Suffolk, United Kingdom), salt (Imeos Enterprises, Runcorn, Cheshire, UK), SAFPRO 5W dough improver (Lesaffre UK & Ireland Ltd., Worcester, UK), dried yeast (Doves Farm Foods Ltd., Berkshire, UK), and unsalted butter (purchased locally, Dublin, Ireland).

2.2. Preparation of flour

Three types of pea flour were used: raw, germinated and toasted pea flour.

Method for germination: whole yellow peas were cleaned and soaked in distilled water for 24 h, at room temperature (22 °C ± 2). The seeds were then rinsed and patted dry before being spread evenly on trays layered with wet filter paper. The seeds were germinated in an incubator at 30 °C and 95% relative humidity for 24 h. Peas which had not sprouted after this time were discarded. The germinated peas were dried for 72 h at 40 °C.

Method for toasting: de-hulled yellow pea seeds were cleaned and toasted at 180 °C for 20 min in a deck oven (MacPan, Thienne, Italy).

Raw, germinated and toasted peas were milled using a Perten Lab mill 3100 (Perten, Australia), equipped with a 0.5–1.00 mm sieve screen for a particle size range of 500–900 μm.

2.3. Flour and dough properties

2.3.1. Scanning electron microscopy (SEM) of flours

Wheat and pea flour samples were sprinkled onto a carbon adhesive coated stub and sputter coated with chromium. Samples were examined in a Zeiss Supra 40 VP field emission electron microscope (Carl Zeiss, Cambridge, UK) operating at 2 kV. Digital 8-bit TIF images were acquired at a range of magnifications from ×250 to × 5000.

2.3.2. Flour pasting properties

The pasting properties of the wheat flour and flour blends were evaluated using a Rapid Visco Analyser (RVA, Newport Scientific Pty. Ltd., Warriewood Australia). Using the RVA general pasting method and moisture correction equations, the water and sample weights were adjusted to reflect the samples moisture contents. Analysis was carried out in triplicate.

2.3.3. Dough mixing properties

Moisture content of the flours was firstly measured using AACC 14-15 A method (2001). Water absorption, mixing behaviour and dough development time of the wheat flour and pea flour blends were studied using a Mixolab (Chopin Technologies, Paris, France). The Chopin S-test protocol was set up following the manufacturer's instructions. Flour blends were mixed for 30 min at a mixing speed of 80 RPM at 30 °C, with a target torque of 1.1 nm. Flour weight for the test was determined by the flour moisture results and the total dough weight was made up to 75 g with distilled water dispersed by the Mixolab. Analysis was carried out in triplicate.

2.3.4. Fundamental oscillatory rheology

2.3.4.1. Amplitude sweep. All bread doughs were prepared as described in Table 1, excluding the addition of yeast. Rheological measurements were performed on a controlled stress rheometer (Anton Paar GmbH, Graz, Austria) fitted with parallel plates consisting of a 50 mm serrated probe and 50 mm serrated base plate. Approximately 5 g of dough sample was placed onto the base plate, and the upper plate was brought to a gap of 1.025 mm where excess sample was carefully trimmed away. The plate was then lowered to a test gap of 1 mm and testing began. The dough was allowed to rest for 5 min to allow relaxation of residual stresses. The test was carried out with a temperature setting of 25 °C and the whole system was covered using a Peltier hood. Analyses took place between 10^{-3}–10^{2}% strain (γ), to determine linear viscoelastic region of the dough samples; 20 measurements were recorded per sample and results showed that all dough formulations showed a linear region between 0.1 and 10% strain.

2.3.4.2. Frequency sweep. Samples were prepared as for amplitude sweep. Following a rest time of 5 min, the frequency was increased from 0.1 to 10 Hz under a constant strain (0.1%) as previously identified from the amplitude sweep. Storage modulus (G′), loss

Table 1
Formulations used for production of breads made from wheat flour (100%) and wheat and yellow pea flour composites (70:30).

	Wheat control	Raw pea flour	Germinated pea flour	Toasted pea flour
Wheat flour[a]	100	70	70	70
Pea flour	–	30	30	30
Fat	1	1	1	1
Salt	2	2	2	2
Improver	1	1	1	1
Yeast	1.5	1.5	1.5	1.5
Water[b]	65.6	59.5	58	63
Mix time (min)[b]	5.45	6.3	5.45	6

[a] Ingredients listed as g/100 g of total flour used.

[b] As determined by Mixolab measurements.

modulus (G″) and complex modulus (G*) versus frequency values were recorded. The frequency sweep was performed at a temperature of 25 °C and 20 measurements were recorded per sample. Analysis was carried out in triplicate.

2.4. Bread formulation

Preliminary baking trials were carried out using a range of pea flour substitution levels (10–50%) to establish the most appropriate level for dough handling and bread preparation. The aim of the study was to maximise nutrient fortification of the bread and as such, the highest possible flour substitution level was desirable. Preliminary trials revealed that doughs of a suitable consistency for handling and preparation could be achieved at up to 30%. Above this however, doughs became stiff and difficult to handle and mould. Following these trials, three different breads were prepared using a 30% replacement level of wheat flour with raw pea flour (RPF), germinated pea flour (GPF) or toasted pea flour (TPF). Wheat control bread was prepared using 100% strong wheat flour (WF). Breads were prepared according to the formulation in Table 1. Ingredients were mixed to the optimal mixing time (determined by Mixolab®) in a Kenwood mixer with a dough hook attachment. The dough was covered and left to rest for 15 mins at room temperature. The dough was then divided into 60 g pieces, kneaded and moulded before being placed into pup loaf tins (80 mm × 60 mm × 40 mm). The doughs were proofed for 45 mins at 35 °C, 80% relative humidity (Koma CDS sunriser, The Netherlands). Breads were baked for 18 mins in a deck oven (MacPan, Thiene, Italy) at 220 °C/200 °C (top/bottom heat). They were then cooled to room temperature before being stored in polyethylene bags. All breads were prepared in triplicate, i.e. 3 bakes per each type of bread, and 10 loaves were produced per bake.

2.5. Bread characterisation

2.5.1. Loaf dimensions and colour

Specific volume and density of each loaf was measured using the TexVol instrument (BV-L370, Sweden). Loaf weight was recorded and specific volume (ml/g) was calculated. Five loaves from each bake were randomly chosen to calculate the specific volume. Loaf crust and crumb were measured using a Chroma meter CR-410 (Konica Minolta, UK), and expressed using the L^*, a^*, b^* colour scale. Ten readings were taken from the surface and ten readings from centre slices for each bake.

2.5.2. Crumb properties

2.5.2.1. Digital image analysis. Loaf height and crumb structure was measured using the C-Cell Bread Imaging System (Calibre Instruments Ltd., Warrington, UK). Loaves were sliced vertically in the centre, and a slice (1 cm thick) was cut from each half. Four centre slices were measured per bake.

2.5.2.2. Confocal microscopy. Bread samples approximately 5 × 5 × 3 mm thick were resin embedded, sectioned and stained for examination by confocal scanning electron microscopy. Resin sections were triple labelled to show major ingredients: starch, protein and cellulosic material. One drop of 0.1% w/w ethanolic solution of fluorescein isothiocyanate (FITC) was added to the resin section to label starch. After 10 s, the FITC was drained off and replaced with one drop of 0.125% w/w aqueous solution of fluorescent brightener 28 (FB28) to label cellulosic material, and finally one drop of Fast Green FCF to label protein (FG, 0.1% w/w aqueous solution). The sections were rinsed gently with running water and a coverslip placed on top. Stained sections were imaged using a Leica SP5 confocal scanning laser microscope (Leica Microsystems, Mannheim, Germany) fitted with ×20 and ×63 oil immersion objectives. Sequential images were acquired using triple-channel imaging: 405 nm blue diode laser to excite the FB28, 488 nm argon laser excitation for FICT and 633 nm helium-neon for FG. Digital 512 × 512 pixel images were obtained for each separate excitation wavelength and channels were combined and pseudocoloured to show starch (green), protein (red) and cellulosic material (blue).

2.5.2.3. Moisture content. Crumb moisture was calculated using a 2-stage method according to the AACC 44-15A method (2001). The crust was removed from centre slices and the samples were dried at 40 °C for 2 h. The dried sample was ground for 20 s using NutriBullet 600 (Australia) before being passed through a sieve (Endecott test sieve, 1680 μm). Samples (10 g) were completely dried using a Brabender oven (Brabender, Duisberg, Germany) at 130 °C for 60 min. Moisture content was calculated using the following equations:

Moisture 1:

$$\frac{(\text{Sample before drying} - \text{Sample after drying}) * 100}{\text{Sample after drying}} \tag{1}$$

Moisture 2:

$$\text{Moisture\%as measured using the Brabender} \tag{2}$$

Total moisture:

$$\frac{(\text{Moisture 1} + \text{Moisture 2}) - (\text{Moisture 1} * \text{Moisture 2})}{100} \tag{3}$$

Moisture content was carried out in duplicate for each bake, on days 1, 3 and 6.

2.5.2.4. Water activity. A section of the central region of two centre slices was crumbled and the water activity was measured using an Aqua Lab Lite (Decagon Devices, WA, USA). Samples were analysed in triplicate for each bake on days 1, 3 and 6, post-baking.

2.5.2.5. Texture profile analysis (TPA). Crumb texture was assessed by conducting a texture profile analysis using a texture analyser (TA-XT2*i*, Stable Microsystems, Surrey UK), equipped with a 25 kg load cell and a 20 mm cylindrical probe. Pre-test, test and post-test speed were set to 2, 1, and 5 mm/s respectively and compression was set to 40%. TPA was conducted on 4 centre slices (1 cm) per bake, on days 1 (24 h after baking), 3 and 6, post-baking.

2.5.3. Proximate composition

Moisture content of the optimised loaf was determined using the AACC 14-15A method (2001). Ash content was determined using AACC 08-01.01 method (1981). Total nitrogen was determined by the combustion method based on the Dumas principle using a nitrogen analyser (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 (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) (Food Safety Authority of Ireland, 2016).

2.6. Statistical analysis

Samples were analysed in triplicate unless otherwise stated and results 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 $p \leq 0.05$. Where ANOVA indicated significant differences were present, Tukey's pairwise comparison was conducted to identify where sample differences occurred. To identify relationships between flour and dough properties and bread quality, bivariate Pearson's correlation analysis was carried out.

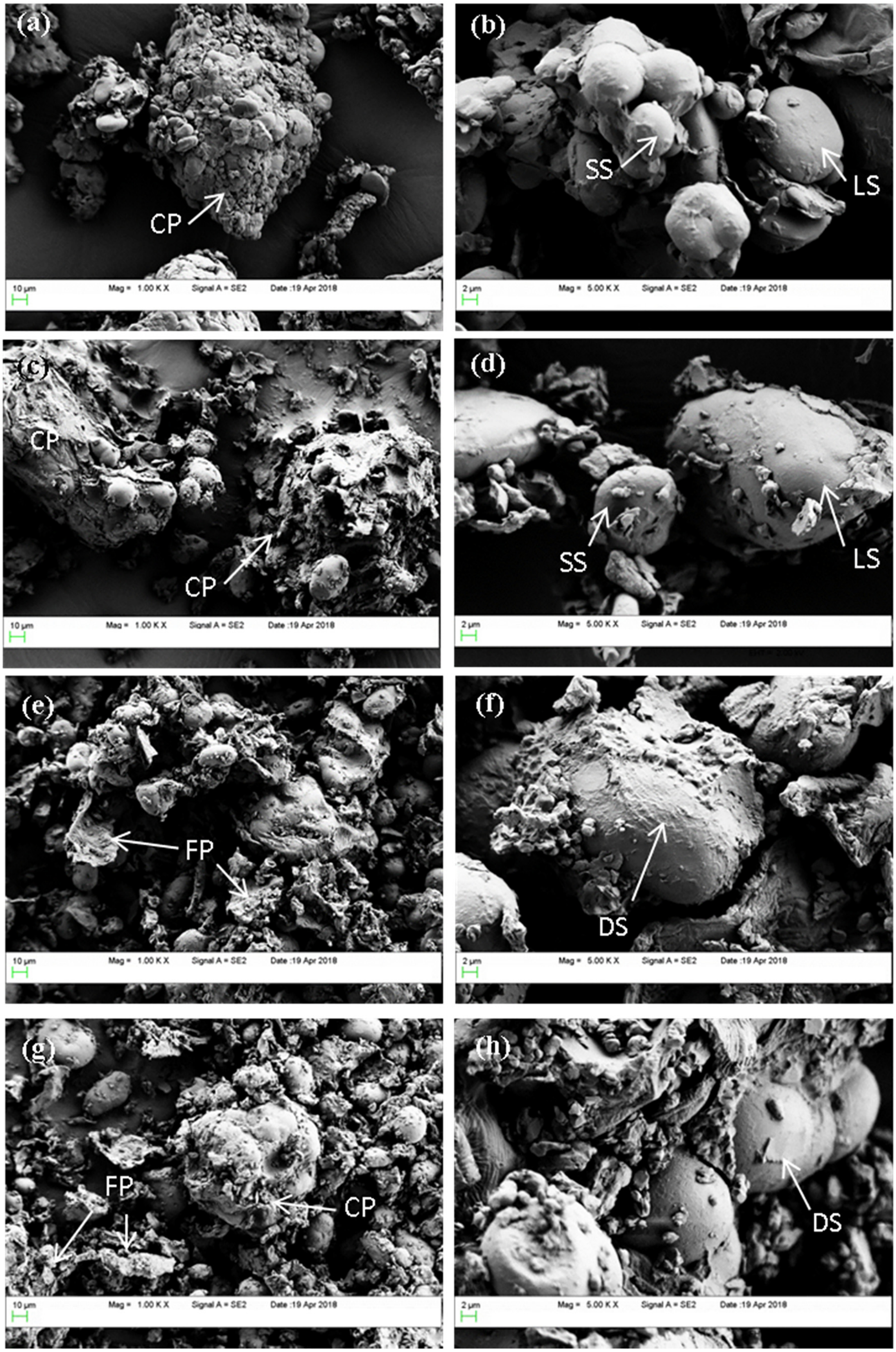

Fig. 1. Scanning electron micrographs of wheat, raw, germinated and toasted pea flours. Wheat flour 1000× (a), 5000× (b); raw pea flour 1000× (c), 5000× (d); germinated pea flour 1000× (e), 5000× (f); toasted pea flour 1000× (g), 5000× (h). CP: continuous protein matrix; FP: fragmented protein matrix; LS: large starch granule; SS: small starch granule; DS: damaged starch.

3. Results and discussion

3.1. Flour properties

3.1.1. Scanning electron microscopy

Scanning electron micrographs of each of the four flours are presented in Fig. 1. The micrographs of wheat flour show highly compact structures of spherical and oval shapes varying in size with smooth surfaces, embedded in a continuous protein network, as is commonly observed in cereal starches (Sakhare, Inamdar, Soumya, Indrani, & Rao, 2014). The pea flours are characterised by larger starch granules which are typically oval in shape and mostly composed of single granules in the pea flours, though there are some compound structures visible. Pulse starches are more often found in the form of single granules, unlike cereal starches, however pea starches have been shown to be composed of both simple and compound structures (Singh, 2011). There is limited research on the structure of pea starches, though size of pulse starches can range from 0.4 to 103 μm and shapes can vary between oval, spherical, elliptical and irregular (Singh, 2011). Other components can be observed bound to the surface which may include carbohydrates, lipids and proteins (Cauvain, 2015), particularly noticeable in Fig. 1(d) and (h). The starch granules of the raw pea flour are characterised by smooth surfaces, compared with those observed in the germinated pea flour where the granule surfaces have become rougher. Similar micrographs of lentil starch were observed by Frias, Fornal, Ring, and Vidal-Valverde (1998) who reported that the surface of the starch granules became more eroded with continued germination of the seeds causing granule degradation.

While the protein matrix in the wheat flours can be observed as a continuous network of spherical protein bodies, the protein matrix in pea flours appears less uniform. Following germination, the protein network of pea flour appeared more fragmented as a result of proteolytic enzyme activity. However, as the peas were germinated for just 24 h, the alteration to the protein network was less than that observed by Frias et al. (1998) and Moongngarm (2011), who reported continued destruction of the protein network using a germination time of 24–144 h. Fig. 1(f) and (h) also shows an increase in adherence of compounds to the surface of the starch granules in the germinated and toasted samples which was likely due to the protein denaturation following processing (Frias et al., 1998).

3.1.2. Flour pasting properties

Significant differences were observed for the pasting profiles across all of the samples studied (Table 2). Starch is a crucial component in developing bread dough, specifically in the formation of the starch-protein matrix. Pasting behaviour is affected by starch size, structure and distribution, amylose, protein and lipid contents, as well as water binding capacity (Wu et al., 2013). Changes to the pasting properties can affect the eating quality of the final bread product, particularly loaf volume and crumb density which are dependent on starch gelatinisation as well as protein gelation and aggregation (Alvarez-Jubete, Auty, Arendt, & Gallagher, 2010). The botanical source of starch is also an important factor in pasting behaviour and may have just as much influence on pasting properties as the gluten proteins and in turn, on loaf properties (Eliasson, 2003).

The toasted pea flour blend had the highest peak viscosity and the shortest peak time, suggesting increased gel strength compared with the other flours ($p < 0.05$). The lower starch content of pea flour compared with wheat flour, has previously been associated with reduced viscosity (Chung, Liu, Hoover, Warkentin, & Vandenberg, 2008). However, protein denaturation which may have occurred during the toasting process, can increase gel forming abilities of flours, explaining the high peak and trough viscosity of the toasted pea flour blend (Morad, Leung, Hsu, & Finney, 1980). Increased gel forming abilities of pea flour following toasting may assist in developing and setting the gas network during proofing and baking, improving the eating quality of the resulting bread (Alvarez-Jubete et al., 2010). There may also be nutritional benefits associated with a reduction in available starch, including a proportional increase in resistant starch and dietary fibres which may induce a lower glycaemic index (Fares & Menga, 2012). Germination of the peas reduced final viscosity of the resulting flour blend ($p < 0.01$), which may have been caused by starch degradation and a reduction in amylose content due to enzymatic activity during germination (Morad et al., 1980). Germination has also been shown to increase the dietary fibre content of pulses (Benítez et al., 2013) which can dilute the starch content of the flour and further reduce the viscosity by competing with the starch for available water (O'Shea, Doran, Auty, Arendt, & Gallagher, 2013).

The raw pea flour blend had a significantly lower breakdown value than all other samples. This implies an increased ability of the flour blend to withstand heating and shear stress, and may be the result of the higher amylose content found in raw pea flours (Singh, Kaur, Rana, & Sharma, 2010). Pulse starches can contain anywhere from 24 to 65% amylose, compared with wheat which contains approximately 25% (Singh, 2011). The reduction in amylose content following germination may have resulted in the higher breakdown value observed in the germinated pea flour blend.

The setback is calculated as the difference between the trough viscosity and final viscosity and may correlate with the final texture of a product. Germinating and toasting reduced the setback of the pea flour blends ($p < 0.001$). This may have been a result of a reduced amylose-amylopectin ratio in these flours which and can affect the setting of the crumb structure and the loaf volume (Alvarez-Jubete et al., 2010).

3.1.3. Dough mixing properties

Dough mixing properties as measured by Mixolab® are presented in Table 3. Water absorption plays a crucial role in hydration and development of the gluten network and can have a significant impact on the final quality of the bread. The wheat-pea flour blends had lower water absorption (%) than the wheat flour control ($p < 0.001$). The germination process significantly reduced the water absorption to 57.9%. The toasting process however, increased it from 59.5% in the raw pea flour blend, to 62.9%. It was expected that the increased protein content, enzymatic activity and the presence of damaged starch would have

Table 2
Starch pasting properties (RVA) of wheat flour (100%) and wheat and yellow pea flour composites (70:30).

	PV[A]	TV	BD	FV	SB	P_{time}	P_{temp}
WF	1607 ± 20^{b}	818 ± 12^{b}	789 ± 10^{a}	1912 ± 48^{b}	1094 ± 35^{a}	5.65 ± 0.04^{a}	85.05 ± 1.23^{a}
RPF	1561 ± 19^{b}	976 ± 18^{a}	585 ± 17^{c}	2058 ± 11^{a}	1082 ± 7^{a}	5.47 ± 0.07^{b}	74.30 ± 2.15^{b}
GPF	1537 ± 59^{b}	815 ± 23^{b}	722 ± 36^{b}	1740 ± 43^{c}	925 ± 22^{b}	5.27 ± 0.07^{c}	73.70 ± 0.95^{b}
TPF	1711 ± 6^{a}	975 ± 6^{a}	736 ± 11^{ab}	1890 ± 15^{b}	914 ± 20^{b}	5.31 ± 0.03^{c}	73.40 ± 0.8^{b}

WF: wheat flour; RPF: raw yellow pea flour; GPF: germinated yellow pea flour; TPF: toasted yellow pea flour.
PV peak viscosity; TV trough viscosity; BD breakdown (PV − TV); FV final viscosity; SB setback (FV − TV); P_{time} peak time (min); P_{temp} pasting temperature (°C).
Data presented as means ± standard deviation from triplicate analysis.
$^{a–d}$Values followed by different superscripts in the same column are significantly different ($p < 0.05$).
[A] Results are displayed as viscosity in centipoise (cP).

Table 3
Dough properties from Mixolab of wheat flour (100%) and wheat and yellow pea flour composites (70:30).

	Moisture content %[A]	Water absorption %	Development (min)	Stability (min)	Weakening (F.U.)[B]
WF	12.7^{a}	65.54 ± 0.04^{a}	4.75 ± 0.35	11.5 ± 0.71^{a}	46 ± 2.8^{d}
RPF	12.1^{a}	59.49 ± 0.13^{c}	5.5 ± 0	3.5 ± 0^{bc}	71.5 ± 4.9^{c}
GPF	11.1^{ab}	57.94 ± 0.09^{d}	4.75 ± 0.35	2.25 ± 0.35^{c}	98 ± 0^{b}
TPF	9.2^{b}	62.92 ± 0.20^{b}	5 ± 0	4 ± 0^{b}	114.5 ± 2.1^{a}

WF: wheat flour; RPF: raw yellow pea flour; GPF: germinated yellow pea flour; TPF: toasted yellow pea flour.
Data presented as means ± standard deviation from triplicate analysis.
$^{a–d}$Values followed by different superscripts in the same column are significantly different ($p < 0.05$).
[A] Moisture content determined with AACC method 11-15A and used to calculate flour weight required for test.
[B] Farinograph units.

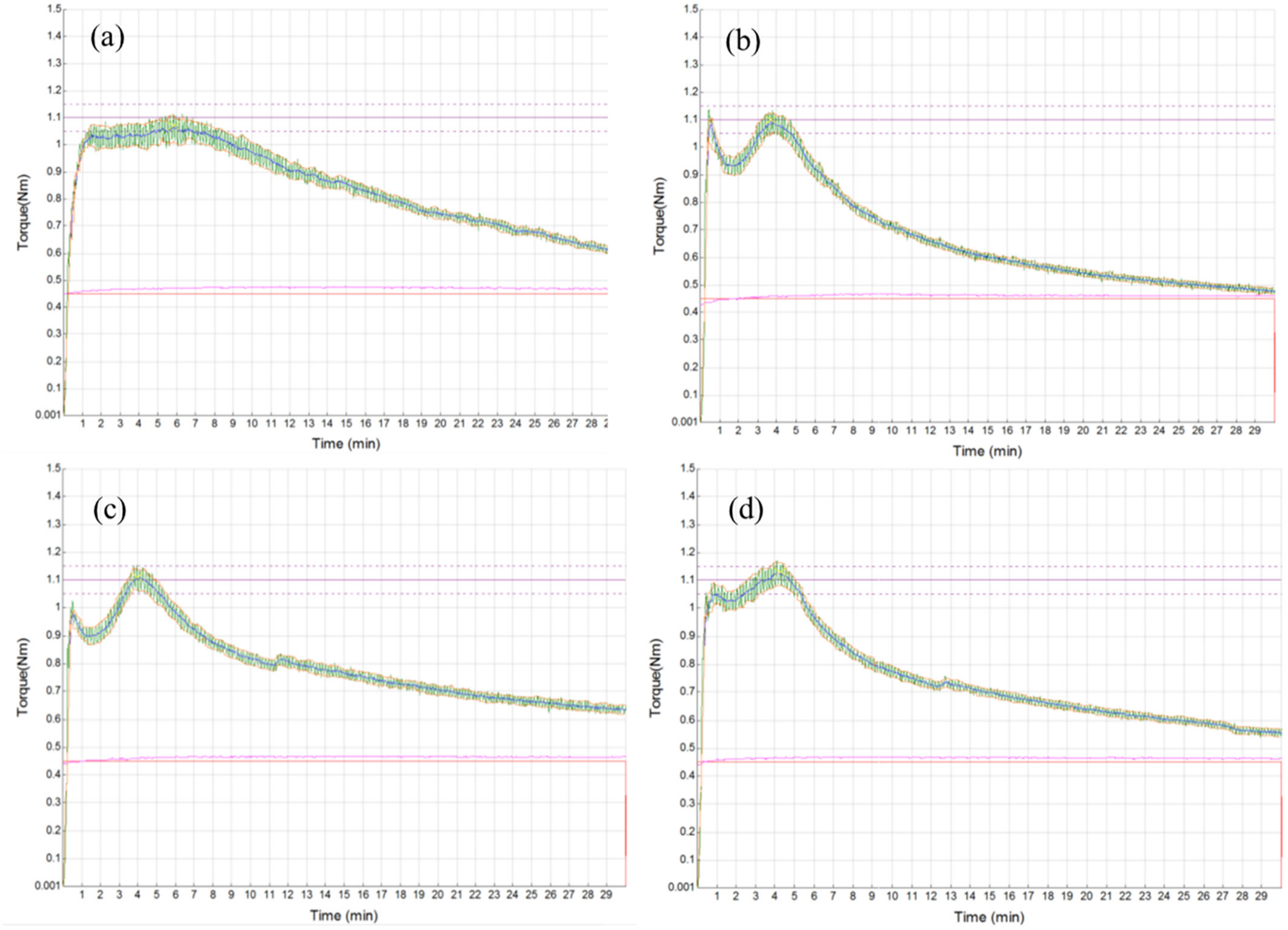

Fig. 2. Mixolab® curves of wheat flour (100%) and wheat and yellow pea flour composites (70:30), as measured using Chopin S test. (a) Wheat flour; (b) wheat-raw pea flour; (c) wheat-germinated pea flour; (d) wheat-toasted pea flour.

increased the water absorption in the pea flour blends, particularly following germination (Hallén, İbanoğlu, & Ainsworth, 2004; Sadowska et al., 2003). However, the loss of starch during germination, as well as protein structural changes, may have had contrasting effects on the water absorption (Maninder, Sandhu, & Singh, 2007). Protein denaturation and starch gelatinisation, following the toasting process, may have resulted in the increase in water absorption, compared to using raw pea flour (Hallén et al., 2004) (Fig. 2).

The development time was not significantly increased following the addition of all pea flours indicating that the initial formation of the gluten network was not adversely affected. However, dough stability and resistance to mechanical mixing was reduced following the addition of all pea flours ($p < 0.001$). The wheat flour dough exhibited the highest stability, indicating the formation of a stable gluten network, which is to be expected for wheat dough. Addition of pea flour interrupted the starch-protein matrix, which can decrease dough elasticity and cause a weakening of the dough during continued mixing.

Sadowska et al. (2003) also observed a reduction in dough stability with increasing levels of pea flour. However the authors reported an increase in dough stability following germination of peas. The reduction in stability following germination in the current study may have been caused by a reduction in starch content or the presence of proteolytic enzymes (Hallén et al., 2004). The authors also suggested that thermal treatments may inactivate proteolytic enzymes and increase the stability of doughs supplemented with pulse flours. This may account for the moderate increase in dough stability time observed

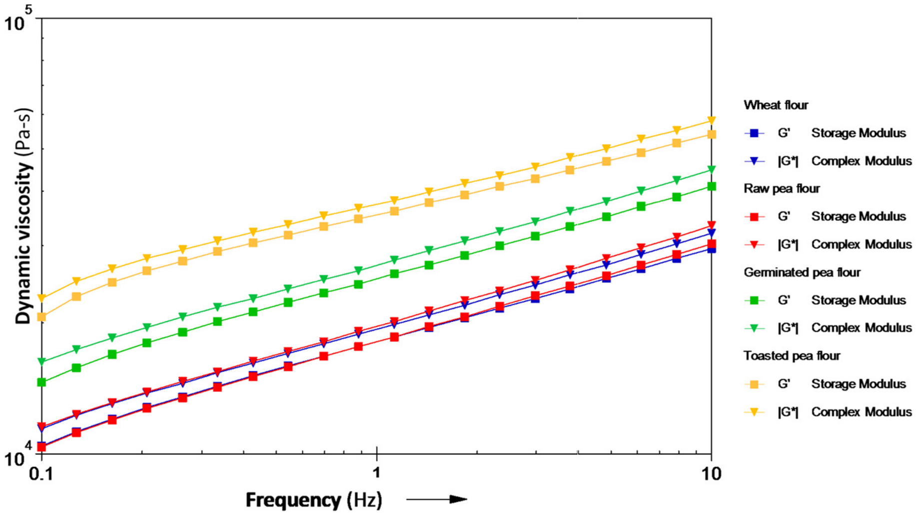

Fig. 3. Frequency sweep of wheat flour (100%) and wheat and yellow pea flour composites (70:30).

following toasting of peas, compared with the raw pea flour.

3.1.4. Fundamental oscillatory rheology

Rheological properties of dough can determine bread quality and texture. Bread dough must have sufficient elasticity to allow for the formation of the gas network, and be strong enough to retain the bubble structure during proofing and baking (Dobraszczyk, 2003). Oscillatory testing was carried out to observe the effects of pea flour on the viscoelastic properties of bread dough. While addition of raw pea flour at 30% had no impact on the rheological properties of the dough, the germination and toasting processes significantly affected storage, loss and complex moduli (Fig. 3). The storage modulus refers to the energy stored in the material after oscillation is removed and provides a measure of dough elasticity while the loss modulus refers to the energy lost during oscillation, and is an indicator of the viscoelastic properties of the dough (Sullivan, O'Flaherty, Brunton, Arendt, & Gallagher, 2011). Storage modulus (G′) was higher than loss modulus (G″) for all doughs, indicating elastic-like behaviour. Both storage and loss moduli were increased by germination and toasting of the peas ($p < 0.001$). The complex modulus (G*), a measure of the overall stiffness or firmness of the bread dough, is calculated based on the ratio of storage and loss moduli. A simultaneous increase in storage and loss moduli therefore increases the complex moduli and thus indicates an increase in dough stiffness (Dobraszczyk, 2003). An increase in denatured proteins, following both processing treatments, may have increased competition for water binding sites in the dough which interferes with the continuous starch-protein network. This network provides the dough with elasticity and this may explain why doughs with processed pea flours had increased stiffness (Sullivan et al., 2011).

3.2. Bread characteristics

3.2.1. Loaf dimensions and colour

There were no significant effects observed for loaf weight or bake loss between the wheat breads and the pea flour composite breads (data not shown). Specific volume decreased in order: WF > TYP > RYP > GYP (Table 4). High specific volume is a desirable attribute as it implies a higher crumb porosity which in turn is associated with freshness (Cauvain, 2015). Addition of raw and toasted pea flour did not significantly reduce loaf specific volume. These results indicate that formation and stabilisation of the gas network during proofing and baking was not adversely affected following wheat flour substitution. Both loaf height and specific volume were reduced following the addition of germinated pea flour however ($p < 0.05$). The specific volume was comparable to that that reported by Mondor et al. (2014) following addition of both raw and germinated pea flour at just a 10% substitution level. It was anticipated that increased protein solubility as a result of germination would improve foaming and emulsifying activity of the pea flours and increase specific volume (Ouazib et al., 2016). In the current study however, the reduction in specific volume may have been a result of the lower water absorption capacity following the loss of starch, leading to a reduction in emulsifying activity and consequently lower specific volume (Benítez et al., 2013). Additionally, germination of the peas produced loaves with a denser crumb. Crumb density increased in the order WF > TYP > RYP > GYP. This increase in density can be also be attributed to the reduced emulsifying and foaming activity of the flours, coupled with the reduced water content of the dough (Benítez et al., 2013).

Loaf crust and crumb properties are presented in Table 4. The L^* value (brightness), was significantly lower for the crust of all breads made with pea flour compared with the wheat flour bread ($p < 0.01$), indicating a darker appearance. Reduction in L^* values has been observed previously in cereal-based products following the addition of pea flours, and can be attributed to the increase in Maillard–browning reactions following the increase in protein content (Millar et al., 2017). Crumb lightness was not affected by the addition of yellow pea flour.

3.2.2. Crumb grain properties

3.2.2.1. Digital image analysis. Digital images of the crumb structure obtained using the C-Cell 2-D imaging software are presented in Fig. 4. The cell structure in bread is largely responsible for appearance and textural properties and digital image analysis offers an objective analysis of the crumb properties, complementary to that of subjective visual and sensory methods. Bread is a porous structure whose final volume is comprised of approximately 70% gas produced during proofing and stabilised within the starch-protein network (Mills, Salt, Jenkins, Skeggs, & Wilde, 2004). The stabilisation of this gas network and the cell structure of the final product are important factors in assessing the quality of white bread (Gonzales-Barron & Butler, 2004).

Cell diameter was between 1.5 and 2 mm for all loaves (Table 5). Cell size is a key indicator carbon dioxide bubbles captured during proofing and is believed to have a significant effect on the crumb texture and sensory properties of bread. Bread with a small cell size can indicate a close crumb structure, resulting in a dense loaf, while a large a cell size, indicates an open crumb structure which can lead to a coarse texture (Gonzales-Barron & Butler, 2005). There were no significant differences found in cell size and Fig. 4 shows a relatively uniform grain in all loaves. This was supported by the non-uniformity value calculated

Table 4

Physical dimensions, crust and crumb colour of breads made from wheat flour (100%) and wheat and yellow pea flour composites (70:30).

	WF	RPF	GPF	TPF
Loaf height (mm)	57.90 ± 2.69^{a}	49.91 ± 1.25^{a}	47.70 ± 3.99^{b}	51.48 ± 1.96^{a}
Specific volume (cm^3/g)	3.74 ± 0.21^{a}	3.01 ± 0.14^{ab}	2.3 ± 0.76^{b}	3.04 ± 0.13^{ab}
Density (g/cm^3)	0.27 ± 0.02^{b}	0.34 ± 0.02^{ab}	0.36 ± 0.04^{a}	0.33 ± 0.01^{ab}
Crust colour				
L*	55.13 ± 1.76^{a}	41.89 ± 2.43^{b}	36.33 ± 0.75^{b}	42.84 ± 2.9^{b}
a*	16.02 ± 0.56^{c}	18.43 ± 0.61^{ab}	16.88 ± 0.46^{bc}	19.61 ± 0.7^{a}
b*	34.39 ± 0.64^{a}	24.66 ± 2.62^{b}	18.01 ± 1.25^{c}	27.43 ± 2.06^{b}
Crumb colour				
L*	69.59 ± 1.78^{a}	71.54 ± 0.88^{a}	71.76 ± 1.93^{a}	70.99 ± 0.58^{a}
a*	0.21 ± 0.13^{c}	0.3 ± 0.21^{c}	1.56 ± 0.20^{a}	1.21 ± 0.21^{b}
b*	14.23 ± 0.2^{d}	19.33 ± 0.17^{c}	20.26 ± 0.3^{b}	23.31 ± 0.27^{a}

WF: wheat flour; RPF: raw yellow pea flour; GPF: germinated yellow pea flour; TPF: toasted yellow pea flour.

Data presented as means ± standard deviation from triplicate analysis.

$^{a–d}$Values followed by different superscripts in the same row are significantly different ($p < 0.05$).

by the C-Cell software, which was below 2 for all loaves. Sadowska et al. (2003) observed a significant increase in crumb porosity and pore wall failure which the authors attribute to the larger starch granules present in pea flour. While the starch granules were shown to be bigger in the pea flours (Section 3.1.1), this did not appear to have negative effects on the crumb structure in the current study and results indicate that crumb structure was maintained following addition of all pea flours.

While there were no significant changes to cell number, slice area was significantly reduced following the addition of germination pea flour ($p < 0.05$), correlating with the reduction in loaf specific volume ($r^2 = 0.8856$). This resulted in an increased number of cells, per slice area in the loaves formulated using germinated pea flour which is indicative of a denser crumb structure (Gonzales-Barron & Butler, 2005).

3.2.2.2. Confocal laser scanning microscopy. Confocal images of the bread crumb are presented in Fig. 4. The food components are labelled starch: green; protein: red; cellulosic material: blue. The micrographs all show an uneven distribution of starch and protein which is common in cereal based foods such as bread (Dürrenberger, Handschin, Conde-Petit, & Escher, 2001). In Fig. 5(a) a continuous protein matrix can be seen surrounded by the starch network, while smaller protein molecules can be identified embedded amongst the starch network. Increased starch gelatinisation has occurred in the breads formulated using germinated and toasted pea flour blends. This can be seen in Fig. 5(c) and (d), where some of the starch granules have lost their original structure. In Fig. 5(d), the protein matrix can be observed surrounding the starch, rather than embedded within the network. This is possibly due to the gelatinisation of the starch granules interrupting the formation of the protein matrix during cooking (Dürrenberger et al., 2001).

The ratio of starch-protein content appears to be reduced in the bread following the addition of germinated pea flour. This supports the previous results indicating a possible reduction in total starch content following germination of the peas. There is a noticeable increase in cellulosic material in the wheat-pea flour breads which may have

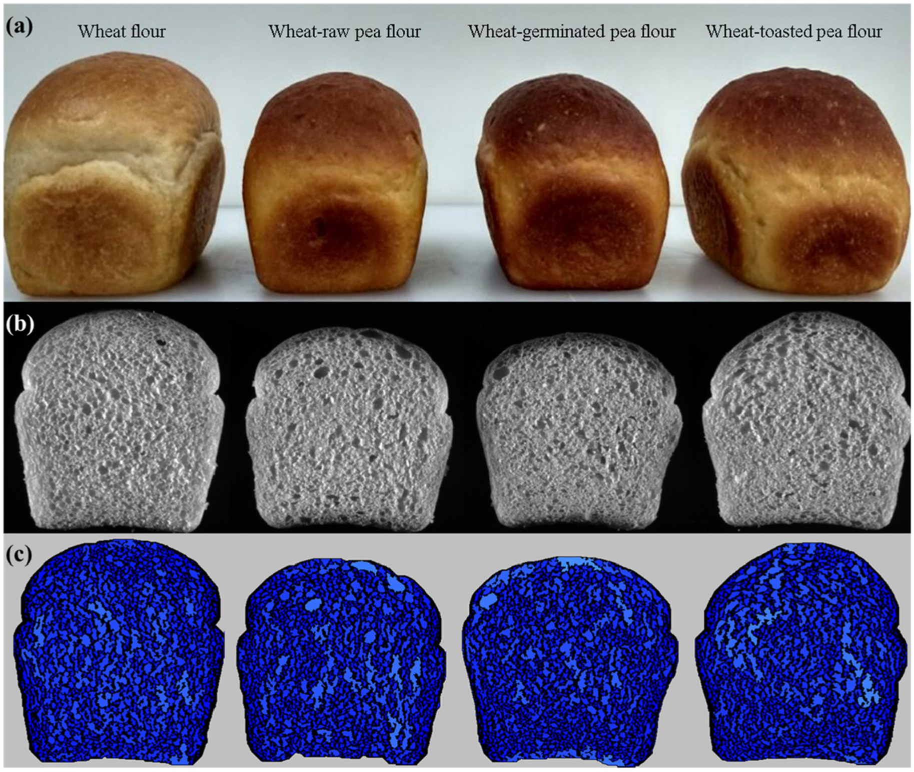

Fig. 4. Images of breads made from of wheat flour (100%) and wheat and yellow pea flour composites (70:30); (a): pup loaves; (b): centre slices; (c): cell structure.

Table 5
Crumb structure (digital image analysis) and texture properties (texture profile analysis) of breads made from wheat flour (100%) and wheat and yellow pea flour composites (70:30).

	WF	RPF	GPF	TPF
Cell diameter (mm)	1.8 ± 0.14	1.52 ± 0.03	1.52 ± 0.17	1.62 ± 0.08
Slice area (mm^2)	2531 ± 97^a	2159 ± 66^{ab}	2071 ± 181^b	2227 ± 76^{ab}
Cell number	1883 ± 109	1799 ± 19	1819 ± 55	1868 ± 131
Moisture content (%)	43.1 ± 1.4^a	40.2 ± 0.5^b	39.2 ± 0.6^b	39.8 ± 0.4^b
A_w	0.929 ± 0.019	0.913 ± 0.010	0.915 ± 0.016	0.912 ± 0.012
Hardness (N)	3.25 ± 0.63^b	8.19 ± 0.83^{ab}	11.98 ± 3.99^a	8.12 ± 0.31^{ab}
Springiness	0.947 ± 0.004^a	0.898 ± 0.010^b	0.885 ± 0.020^b	0.903 ± 0.015^b
Cohesiveness	0.726 ± 0.036^a	0.568 ± 0.016^b	0.543 ± 0.016^b	0.571 ± 0.027^b
Resilience	0.378 ± 0.044^a	0.250 ± 0.015^b	0.227 ± 0.014^b	0.241 ± 0.232^b

WF: wheat flour; RPF: raw yellow pea flour; GPF: germinated yellow pea flour; TPF: toasted yellow pea flour.
Data presented as means ± standard deviation from triplicate analysis.
$^{a–d}$Values followed by different superscripts in the row are significantly different ($p < 0.05$).

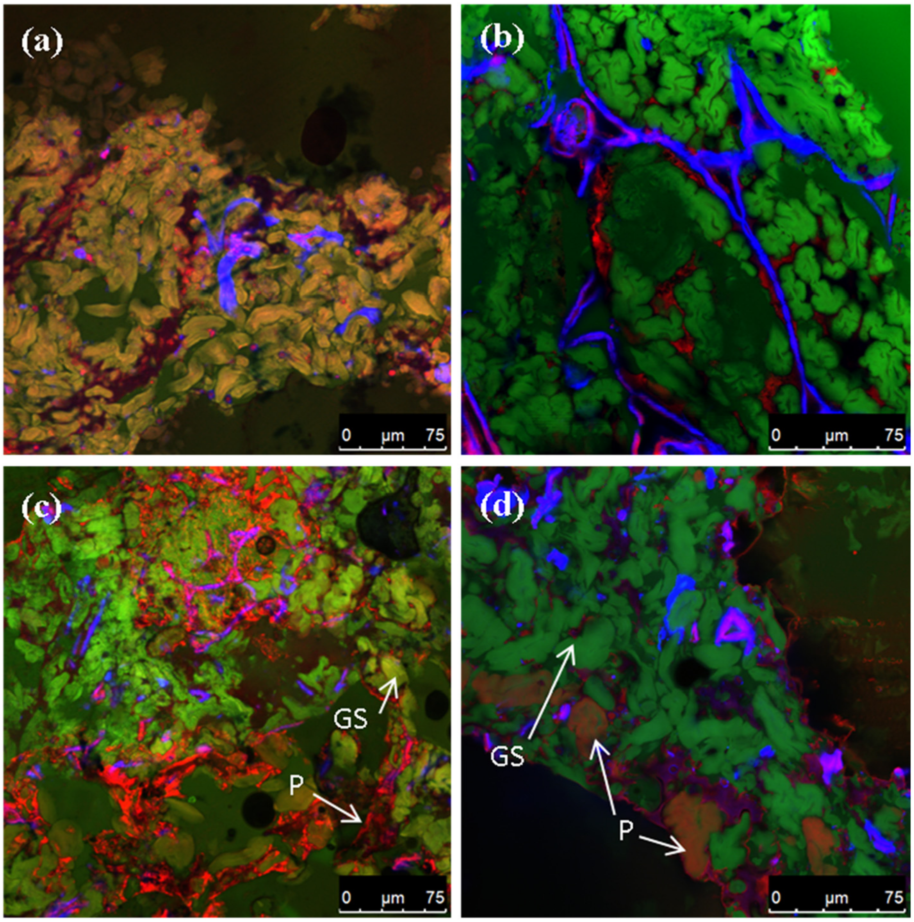

Fig. 5. Confocal laser scanning micrographs of breads made from of wheat flour (100%) and wheat and yellow pea flour composites (70:30). (a) Wheat flour control; (b) raw pea flour blend; (c) germinated pea flour blend; (d) toasted pea flour blend. Food components are labelled starch: green; protein: red; cellulosic material: blue. P: protein; GS: gelatinised starch.

played a role in such properties as water absorption and loaf volume. The increase in cellulosic material can interrupt the formation and stabilisation of a gas network during the bread proofing (O'Shea et al., 2013).

3.2.2.3. Moisture content and water activity. Crumb moisture content and water activity (A_w), as measured 24 h after baking, are presented in Table 5. Measurements were also taken on days 3 and 6 post-baking and the data was analysed for interactive effects between the treatments (flour blend) and time (days). All loaves formulated with pea flour blends had lower crumb moisture compared with the wheat control ($p < 0.05$). This can be partly attributed to the reduced water required for dough formulation in these breads. Crumb moisture remained stable for 3 days, before decreasing between days 3 and 6 ($p < 0.01$). There were no interactive effects, indicating the addition of pea flour had no effect on the change in moisture content over the testing period (data not shown).

While there were no differences observed in crumb A_w 24 hour post-baking, over the full 6-day testing period, addition of all pea flours reduced the average crumb A_w. There was a reduction in A_w from day 1 to day 6; however, there were no interactive effects, indicating that the rate of reduction in A_w was not affected by the flour used (data not shown).

3.2.2.4. Texture profile analysis (TPA). TPA revealed that the texture properties of the bread were significantly affected by the addition of pea flour. Crumb hardness increased in the order WF < TPF < RPF < GPF, Table 5. While toasting did not

Table 6
Proximate composition of breads made from wheat flour (100%) and wheat and yellow pea flour composites (70:30).

g/100 g	WF	RPF	GPF	TPF
Moisture	33.3	35.1	32.9	34.9
Ash	2.1	2.4	2.4	2.3
Protein	8.4[a]	10.1[b]	10.8[b]	10.4[b]
Fat	1.9	1.8	1.9	1.8
Carbohydrate	54.3	50.6	52.1	50.7
Total kcal/100g[A]	267.9	259.4	253.4	260.2
% energy from protein[B]	12.5[a]	15.6[b]	17[b]	15.9[b]

WF: wheat flour; RPF: raw yellow pea flour; GPF: germinated yellow pea flour; TPF: toasted yellow pea flour.
Data presented as means ± standard deviation from triplicate analysis.
[a–d]Values followed by different superscripts in the same row are significantly different ($p < 0.05$).
[A] Calculated at 4 kcal/g carbohydrate; 4 kcal/g protein; 9 kcal/g fat (Food Safety Authority of Ireland, 2016).
[B] kcal provided by protein calculated as % of total kcal (Food Safety Authority of Ireland, 2016).

significantly affect crumb hardness, germination did result in breads with an increased crumb hardness ($p < 0.05$). Crumb springiness, cohesiveness, and resilience were also reduced by the addition of all pea flour ($p < 0.01$). The increase in crumb hardness and reduction in crumb springiness and cohesiveness can be caused by the reduction water absorption of the dough, and subsequent reduction in moisture content of the crumb. Similar results have been observed by Ouazib et al. (2016) and Sadowska et al. (2003). There is little to no information available however, on the effects on crumb texture of white bread following substitution levels above 12–15%.

There were no changes in crumb hardness up to day 3 in all loaves. At day 6, crumb hardness was increased in loaves formulated with raw and germinated pea ($p < 0.05$) while there were no significant changes observed in the wheat control or that produced with toasted pea flour. Crumb springiness, cohesiveness and resilience reduced significantly from day 1 to day 6 in all breads ($p < 0.01$), with no effects caused by the flour used.

3.2.3. Proximate composition

Proximate composition analysis, presented in Table 6, revealed that the addition of pea flour significantly increased the protein content from 8.4% in the control, to 10.1–10.8 in breads formulated with pea flour. The toasting and germination process did not affect the protein content of the resulting breads. The total % energy (kcal) provided by protein was also increased from 12.5 to 15.6–17%.

3.3. Correlation analysis

A bivariate Pearson's correlation analysis revealed significant relationships between dough properties and the characteristics of the final bread loaves. Loaf specific volume and loaf density were significantly affected by water absorption ($r^2 = 0.74$, $p < 0.05$ and $r^2 = -0.86$, $p < 0.01$ respectively), and dough stability ($r^2 = 0.70$, $p < 0.05$ and $r^2 = -0.92$, $p < 0.01$ respectively). The reduction in water content used in dough mixing may have reduced the foaming activity of the flour. This can limit the formation of the gas network and consequently reduce the final volume of the loaf. The instability of the dough causes further failure of the gas network resulting in a less porous loaf with increased density (Benítez et al., 2013).

Crumb texture properties were also significantly affected by changes in water absorption and dough stability. Reduced water absorption has previously been associated with increased crumb hardness, and reduced crumb cohesiveness (Ouazib et al., 2016). In the current study relationships were observed between water absorption and crumb hardness ($r^2 = -0.86$, $p < 0.01$), springiness ($r^2 = 0.90$, $p < 0.01$), resilience ($r^2 = 0.77$, $p < 0.05$) and cohesiveness ($r^2 = 0.82$, $p < 0.05$). Increasing the water absorption of the flour, which occurred following toasting the peas, can improve emulsifying activities and increase the moisture content of the crumb grain, producing loaves with a softer crumb texture.

Dough stability was correlated with crumb hardness ($r^2 = -0.94$, $p < 0.001$), springiness ($r^2 = 0.93$, $p < 0.001$), resilience ($r^2 = 0.87$, $p < 0.01$) and cohesiveness ($r^2 = 0.93$, $p < 0.001$). The denser loaf produced as a result of reduced dough stability has a more closed crumb structure which is brittle, causing an increased crumb hardness. Highly aerated doughs are more desirable as they produce loaves with a more porous crumb structure which increases crumb springiness, cohesiveness and resilience, and reduces crumb hardness (Dürrenberger et al., 2001). This is supported by the relationships observed between cell volume and crumb hardness ($r^2 = -0.80$, $p < 0.001$), crumb springiness ($r^2 = 0.70$, $p < 0.05$) and crumb resilience ($r^2 = 0.68$, $p < 0.05$).

4. Conclusions

The effect of 30% pea flour (raw, germinated and toasted) substitution of wheat flour on dough rheological properties and baking characteristics of white bread were investigated. The toasting process yielded pea flour with improved gel forming abilities compared with germinated pea flour. This demonstrates a potential in using thermal treatments to improve the functionality of pea flours, increasing their range of applications in food innovation. Doughs formulated using raw pea flour had comparable viscoelastic properties to that of the wheat flour control, indicating adequate development of the gluten network.

Loaf characteristics, including specific volume, density and crumb texture were all affected by changes to the dough mixing properties, following the addition of the pea flours. Substitution with raw and germinated pea flour reduced water absorption; however the toasting process increased water absorption of the pea flour blend. This resulted in loaves with comparable specific volume and density to the wheat flour control. This highlights that such thermal processing methods may be applied to manipulate dough mixing properties to improve loaf quality.

The protein content was significantly increased following the substitution of pea flour at 30%, highlighting the potential of pea flour to increase the protein content of breads and other cereal foods. There is currently little to no research on the use of high-protein pea flours in bakery products above a substitution level of 10–15%, without detrimental effects on the quality of the final product. Results from the current study clearly demonstrate that high quality bread can be achieved at a flour substitution level of up to 30% of pea flour, thereby providing the consumer with alternative bread with enhanced protein and other nutritive properties. This may assist in achieving adequate protein in diets which are primarily cereal based.

This preliminary study indicated that bread formulated with toasted pea flour can result in similar properties to wheat flour bread. These results imply potential for a product with consumer acceptability, though they cannot fully indicate the potential success of such a product alone. Currently, comprehensive sensory analysis is being carried out to ensure a positive sensory profile of the bread and ultimately a high level of consumer acceptability.

Declaration of Competing Interest

I have no conflicts of interest to disclose. I confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

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