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Effect on germination time and temperature on techno-functional properties and protein solubility of pigeon pea (*Cajanus cajan*) flour

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RESEARCH ARTICLE

Abstract

Germination is a natural low-cost bioprocessing technique that can be used to enhance the functionality of food grains. The present study evaluates the effect of germination time and temperature on the functional characteristics and protein solubility of germinated pigeon pea flour. Germinated pigeon pea flour exhibited a reduction in bulk density, water absorption and swelling power, as well as an enhanced gelling consistency and oil absorption capacity in comparison to raw flour due to enzymatic modification of starch and proteins. Germination for 48 h at 35 °C decreased the paste clarity of processed flour, while synereis of flour was increased. Prolonged germination time positively increased the protein solubility and least gelation concentration of pigeon pea flour. Raw flour showed the lowest emulsion and foaming properties, while an increase in germination conditions increased the emulsifying and foaming properties of the flour.

Keywords: germination, pigeon pea, functionality, foaming, emulsifying, protein solubility

1. Introduction

Food legumes are the main dietary energy source in many developing countries due to their low cost, long conservation time and high nutritional value (Ghavidel and Prakash, 2006). Aside being a good source of proteins, they are abundant in starch (22-45%), B complex vitamins, minerals, fibres, resistant starch and phyto-chemicals; thus they make an important contribution to the human diet (Benítez *et al.,* 2013; Siddiq *et al.,* 2010). Pigeon pea (*Cajanus cajan*), also known as Arhar or Red gram, is a rich source of carotene, ascorbic acid, B complex vitamins, calcium, phosphorus and iron, along with high levels of amino acids, such as methionine, lysine and tryptophan. The high protein content of pidgeon peas is considered to compensate the amino acid deficiencies of cereal proteins (Kaushal *et al.,* 2012; Torres *et al.,* 2007). India contributes to 90% of the world pigeon pea production, however, the crop is widely grown and consumed elsewhere in the tropics and semi-arid tropics of the world. Pigeon peas have a potential economic value as a source of high protein (20-26%) (Maninder *et al.,* 2007).

Till date most of the pigeon peas have been used as a vegetable by cooking them with salt and spices. Like other legumes, pigeon peas have also been processed into tempeh, protein concentrate and pigeon pea flour (Wisaniyasa *et al.,* 2015). However, the use of pigeon pea flour has been limited to the preparation of bread (Gayle *et al.,* 1986), pasta (Martinez-Villaluenga *et al.,* 2010; Torres *et al.,* 2007) and biscuits (Tiwari *et al.,* 2011) at relatively low concentrations due to the poor functional properties of the raw pigeon pea flour and the sensory qualities it imparts to the final prepared product.

Germination is a low-cost natural bioprocessing technique that results in enhancing the nutritional value, bioactive components, functional and sensorial properties of the food grains (Singh and Sharma, 2017). Germination results in the modification of protein and starch and accumulation of bioactive components as a result of the action of hydrolytic enzymes. These modified components have a completely different functionality as compared to native flour and can be utilised in small amounts to different food products for a specific purpose (Singh *et al.,* 2017a). Sharma *et al.*

(2019) reported that flour from germinated pigeon peas exhibited higher antioxidant activity, bioactive components and enhanced nutrient digestibility in comparison to raw flour. The functional properties exhibited by legume flour are governed by physicochemical properties of the flour components (protein and starch), their processing behaviour in the food matrix system, size and structure of the macromolecules and their interaction with other food components, such as carbohydrate fibres and fats (Singh *et al.,* 2017b). The modification of the functional properties of the various legumes as result of germination has been studied earlier by Ghavidel and Prakash (2006) for green gram, cowpea, lentil, and Bengal gram, and by Benítez *et al.* (2013) for cowpea and dolichos.

Flour prepared from germinated legumes was not available on the market despite it possessed health promoting components, low antinutritional factors and better sensorial properties. Market value, utilisation and consumers acceptability of germinated pigeon pea flour as functional food ingredient and inexpensive protein source was governed by the techno-functional properties, sensory qualities and protein solubility they impart to the end-product (Maninder *et al.,* 2007; Singh and Sharma, 2017). Therefore, knowledge of the functionality of flour prepared from germinated pigeon pea is desirable. This study was designed to investigate the effect of germination conditions on the selected functional properties and protein solubility of germinated pigeon pea flour for its utilisation in the development of specialty food products with potential health benefits.

2. Materials and methods

Germination of pigeon pea

Pigeon pea (variety AL 201), obtained from Punjab Agricultural University, Ludhiana, was cleaned and steeped in distilled water for 10 h at 25 °C to initiate the germination process. The soaked grains were then spread out thinly on a wet double layered muslin cloth and covered with another water-saturated layer of muslin cloth and allowed to germinate in an incubator (Narang Scintific Works, New Delhi) for 12, 24, 36 and 48 h at 25, 30 and 35 °C. When necessary, water was sprinkled over the muslin cloth. After treatment, grains were taken out and dried to constant weight at 45±2 °C (m.c. 8±2%) in hot air drier. The vegetative portions were separated by rubbing manually. The peas were ground in a super mill (Gibbons Electric, Tollesbury, UK) to produce flour and passed through a 60 BSS mesh screen. Flour was packed and stored at 4 °C until analysed. The non-germinated legume flour served as control.

Functional characteristics

Bulk density

The method by Singh *et al.* (2017a) was employed to determine the bulk density of flour samples and the results were expressed as g/cm^3.

Water/oil absorption capacity and swelling power

The method by Singh *et al.* (2017a) was used to determine the water/oil absorption capacity and swelling power. The gain in weight was used to calculate the water/oil absorption capacity and results were expressed as %. Swelling power was expressed as the ratio of weight of the wet sediment to the weight of dry flour (g/g).

Gel consistency

0.2 g flour sample was wetted in a tube with 0.2 ml 95% ethanol and mixed with 3 ml of 0.1N acetic acid. The suspension was heated for 8 min in a boiling water bath and then allowed to cool down to room temperature for 30 min in upright position. After cooling, the tubes were laid down horizontally for 1 h, and the migration of the gel front was measured to the nearest millimetre (Elkhalifa and Bernhardt, 2013).

Paste clarity

The method described by Singh *et al.* (2017b) was employed to measure paste clarity. Briefly, 1% flour dispersion was heated in a boiling water bath for 30 min with occasional stirring. Then, tubes were removed and cooled at room temperature for 15 min and light transmittance (%T) was measured at 660 nm with a Spectronic-20 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) using distilled water as a blank.

Emulsifying and foaming properties

The method described by Elkhalifa and Bernhardt (2013) was used to measure the emulsifying and foaming properties of the flour samples. The ratio of the height of the emulsion layer to the height of the liquid was recorded to calculate emulsion activity (%). Emulsion stability (%) was calculated as the height of the emulsion layer after heating to the height of the liquid. The foaming capacity was measured by recording the volume of the foam after whipping and expressed as % foaming capacity. The fall in volume of the foam as a function of time for 1 h was recorded as foam stability.

Least gelation concentration

The method of Singh *et al.* (2017a) was followed to measure the least gelation concentration (LGC). Briefly, 2 to 20%, 25% and 30% (w/v) flour suspensions were prepared and heated in a boiling water bath for 1 h. Test tubes were first cooled under tap water and further for 1 h at 4 °C. Tubes were inverted upside-down to check whether the suspensions had formed a gel or not. LGC reflects the critical concentration of flour below which no self-supporting gel is formed.

Freeze-thaw stability (% syneresis)

The method of Elkhalifa and Bernhardt (2013) was employed to measure % syneresis of samples. Briefly, 6% w/v flour suspension was prepared and heated in a boiling water bath for 30 min to form paste. 10 g paste was transferred into previously weighed centrifuge tubes and samples were preserved at 4 °C for 22 h followed by thawing for 90 min at 30 °C. Thawed samples were centrifuged at 3,000 rpm for 15 min and supernatant was decanted and the residue was weighed. % Syneresis was calculated as the ratio of the weight of the liquid decanted to gel weight.

Protein solubility

The method of Singh *et al.* (2017a) was employed to measure protein solubility of the flour. Briefly, 1.0 g of flour sample was mixed with 50 ml of distilled water to prepare a suspension (pH 6.0), which was shaken continuously for 1 h at room temperature. The suspension was then centrifuged for 20 min at 2,000 rpm and supernatant was collected. The supernatant was used to determine the protein solubility of flour and bovine serum albumin was used as a standard to express the results.

Statistical analysis

Germination experiments were performed in duplicate and tests in triplicate. Each value represents mean ± standard deviation of three separate readings. Data obtained from aforesaid tests was subjected to statistical analysis, i.e. analysis of variance (ANOVA). Tukey's post-hoc test was used to describe significant differences in means with $P<0.05$ confidence using SPSS statistical software (version 16.0; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

Bulk density

Bulk density indicates the behaviour of flour in a dry mix and relies upon physicochemical properties, structural characteristics and firmness of flour. Bulk density was significantly ($P<0.05$) decreased by germination time and temperature (Table 1). Control flour exhibited the highest bulk density. Increase in germination time from 12 to 48 h and temperature from 25 to 35 °C significantly ($P<0.05$) decreased the bulk density of the flour by 4.59-18.39% due to reduction in heaviness and dispersibility of the flour. During germination, structural modification of starch, protein and

Table 1. Effect of Germination conditions on the bulk density, water and oil absorption capacity and swelling power of pigeon pea flour.[1,2]

Treatment	Temperature (°C)	Time (h)	Bulk density (g/cm^3)	Water absorption capacity (%)	Oil absorption capacity (%)	Swelling power (g/g)
Control	–	–	0.87 ± 0.01^{a}	144.03 ± 0.51^{a}	81.16 ± 0.03^{k}	4.70 ± 0.30^{a}
Soaked	25	10	0.88 ± 0.02^{a}	144.85 ± 0.51^{a}	82.92 ± 0.00^{ijk}	4.64 ± 0.03^{ab}
Germination conditions	25	12	0.83 ± 0.01^{bP}	137.39 ± 0.49^{bP}	83.76 ± 0.30^{ijR}	4.63 ± 0.01^{abP}
		24	0.81 ± 0.02^{bcPQ}	133.00 ± 0.47^{cQ}	84.83 ± 0.20^{ghR}	4.36 ± 0.11^{cdQ}
		36	0.78 ± 0.01^{deQ}	129.40 ± 0.46^{dR}	88.82 ± 0.87^{deQ}	4.15 ± 0.08^{efQ}
		48	0.74 ± 0.03^{ghR}	121.94 ± 0.43^{fgS}	92.04 ± 0.13^{cgP}	3.90 ± 0.04^{gR}
	30	12	0.80 ± 0.01^{cP}	131.23 ± 0.47^{cP}	84.52 ± 0.07^{hijR}	4.59 ± 0.01^{bcP}
		24	0.77 ± 0.02^{ePQ}	126.28 ± 0.45^{eQ}	85.74 ± 0.31^{fgR}	4.26 ± 0.06^{dePQ}
		36	0.75 ± 0.03^{fQ}	123.66 ± 0.44^{fR}	91.54 ± 0.44^{cQ}	3.93 ± 0.11^{gPQ}
		48	0.70 ± 0.01^{hiR}	117.74 ± 0.42^{hS}	95.08 ± 0.93^{bP}	3.71 ± 0.29^{hQ}
	35	12	0.78 ± 0.02^{deP}	128.68 ± 0.46^{dP}	87.26 ± 0.35^{efS}	4.25 ± 0.04^{deP}
		24	0.74 ± 0.00^{fPQ}	122.66 ± 0.43^{fQ}	89.62 ± 0.51^{dR}	4.11 ± 0.07^{efP}
		36	0.73 ± 0.01^{ghQ}	120.63 ± 0.43^{gR}	95.91 ± 0.56^{bQ}	3.77 ± 0.02^{hQ}
		48	0.71 ± 0.01^{hiQ}	114.94 ± 0.41^{iS}	97.89 ± 0.65^{aP}	3.21 ± 0.05^{iR}

[1] Values having different superscript letters in a column differ significantly ($P<0.05$) among different treatments.
[2] Values having different capital superscript letters differ significantly ($P<0.05$) for activation time within each temperature.

dietary fibre occurs, resulting in decreased heaviness of the flour particles and as a consequence a lower bulk density (Chinma *et al.,* 2009). Similar results were reported by Ghavidel and Prakash (2006) and Benítez *et al.* (2013) in flours from various germinated legumes. They also reported that flours with a higher bulk density had disadvantages for their utilisation in weaning food formulations. Therefore, use of flour prepared from germinated legumes has an advantage in these formulations due to their lower bulk density. Among various processing techniques opted for the preparation of weaning food formulations with reduced bulk density, germination has been reported to be very useful.

Water absorption capacity

Water absorption capacity (WAC) indicates the maximum quantity of water that flour can absorb. WAC is an important functional property of the flour which enhances the product softness and digestibility. WAC was significantly (P<0.05) affected by germination conditions (Table 1). Control pigeon pea flour had the highest water holding capacity with a WAC of 144%. An increase in germination time from 12 to 48 h at different germination temperatures (25, 30 and 35 °C) significantly (P<0.05) decreased the WAC of flour from 8.05 to 17.92% compared to raw flour. In the present study we found that a prolonged germination time (48 h) with the highest germination temperature (35 °C) lowered the WAC most. During the germination process various hydrolytic enzymes, such as amylases, proteases and fibre degrading enzymes are activated, resulting in the fragmentation and breakdown of starch, protein and fibre content. This leads to a subsequent increase in dextrin and fermentable sugars that provoke the release of water entrapped within the starch granules thereby reducing the WAC (Singh and Sharma, 2017). Singh *et al.* (2017a,b) also reported that an increase in germination time and temperature significantly decreased the WAC in germinated sorghum and brown rice flour.

Oil absorption capacity

Oil absorption capacity (OAC) represents the physical entrapment of oil by flour components. Oil acts as a flavour retainer and enhances the mouth feel of food product. OAC was significantly (P<0.05) influenced by germination conditions. Table 1 shows that germination of pigeon pea resulted in improvement of the OAC as compared to non-germinated flour. Control flour exhibited the lowest OAC (81.16%); this was significantly (P<0.05) increased by 3.20-20.61% with increased germination time and temperature (Table 1). Ghavidel and Prakash (2006) also reported that germination intensified the OAC of flour prepared from green gram, cowpea, lentil and bengal gram. During germination non-polar residues are unmasked by disassociation and partial unfolding of polypeptides chains, thereby increasing the surface availability of lipophillic amino acids that aid in the hydrophobic association of peptide chains and lipids. These hydrophobic associations of protein with fat droplets increase their capacity to absorb and retain more oil (Singh and Sharma, 2017). Legume flour with a high OAC can be helpful in the formulation of meat and bakery foods where OAC is of prime importance (Singh *et al.,* 2017b).

Swelling power

Swelling power represents the ability of flour to swell freely when heated in excess water. Swelling power was significantly (P<0.05) affected by germination conditions (Table 1). Control flour had the highest swelling power of 4.75 g/g, which decreased progressively as a result of germination. Increase in germination time and temperature reduced the swelling power from 4.63 to 3.21 g/g – a reduction of 2.52 to 32.42% as compared to control flour. The results show that maximal swelling occurs closer to their optimum gelatinisation temperature (Table 5), at short germination time when flour components have not yet been degraded to a large extent. Benítez *et al.* (2013) and Wisaniyasa *et al.* (2015) reported similar results in flour prepared from germinated non-conventional legumes and pigeon pea, respectively. During germination, amylase protease enzymes are activated which hydrolyse the starch and protein molecules resulting in the production of low molecular weight components that restrict the ability of the flour to swell during cooking and causing a reduction of the swelling power (Singh *et al.,* 2017b).

Gelling consistency and paste clarity

Germination had a significant positive (P<0.05) effect on gelling consistency (GC), while it exerted a significant (P<0.05) negative effect on paste clarity (PC) of pigeon pea flour (Table 2). Control flour produced thick gels, having a GC of 49.00 mm, while germination resulted in weak gels, with increased GCs from 67.00 to 143.5 mm. Increase in germination time from 12 to 48 h and temperature from 25 to 35 °C increased the consistency of the gels by 53.39 to 141.49% as compared to the control flour. The increase in the GC of germinated flour is due to modification of the grain by amylases and proteases that breakdown the starch and protein matrix and lower the viscosity of the gruels, resulting in the production of thinner gels (Singh *et al.,* 2017b).

Non-germinated flour exhibited the highest PC of 53.65%, while an increase in germination time from 12 to 48 h and temperature from 25 to 35 °C decreased the PC of flour to 17.85-41.05% (Table 2). Teli and Sheikh (2011) reported similar results in germinated and native maize starch. They also reported that when swelling power of germinated starch is lower, its paste clarity (PC) will also be lower. The

Table 2. Effect of germination conditions on gel consistency, paste clarity and emulsifying properties of pigeon pea flour.[1,2]

Treatment	Temperature (°C)	Time (h)	Gel consistency (mm)	Paste clarity (% T)	Emulsifying properties	
					Emulsion activity (%)	Emulsion stability (%)
Control	–	–	49.0±1.41^{h}	53.65±0.07^{a}	49.73±0.69^{j}	42.62±0.44^{hi}
Soaked	25	10	50.5±0.71^{h}	45.25±0.64^{b}	51.00±0.71^{i}	43.92±0.45^{h}
Germination conditions	25	12	67.0±2.83^{gR}	41.05±1.06^{cP}	52.28±0.72^{hS}	44.30±0.45^{hR}
		24	72.5±0.71^{gR}	39.20±1.13^{cdP}	56.10±0.78^{efR}	48.03±0.49^{fgQR}
		36	90.5±2.12^{efQ}	28.80±0.14^{fQ}	67.58±0.94^{cdQ}	51.48±0.53^{efQ}
		48	102.0±1.41^{cdP}	25.80±0.14^{ghR}	70.13±0.97^{bcP}	58.43±0.60^{dP}
	30	12	71.5±3.54^{gR}	36.95±0.07^{deP}	53.55±0.74^{ghS}	46.67±0.48^{gR}
		24	84.0±2.83^{fQ}	27.01±0.56^{fgQ}	57.38±0.80^{eR}	49.73±0.51^{fR}
		36	99.5±2.12^{deP}	24.35±0.07^{hiR}	65.80±0.91^{dQ}	60.47±0.62^{cdQ}
		48	109.5±3.54^{cP}	22.30±0.42^{ijS}	71.40±0.99^{bP}	68.19±0.70^{abP}
	35	12	87.0±2.83^{fS}	35.45±0.07^{eP}	53.55±0.74^{ghR}	47.69±0.49^{gS}
		24	104.5±3.54^{cdR}	20.40±0.14^{jkQR}	67.58±0.94^{cdQ}	59.72±0.61^{cdR}
		36	129.5±2.12^{bQ}	18.88±1.06^{klR}	72.68±1.01^{abP}	66.88±0.68^{bQ}
		48	143.5±2.12^{aP}	17.85±0.67^{lR}	73.95±1.03^{aP}	70.48±0.72^{aP}

[1] Values having different superscript letters in a column differ significantly (P<0.05) among different treatments.
[2] Values having different capital superscript letters differ significantly (P<0.05) for activation time within each temperature.

reduced PC of germinated flour is due to the oxidizing effect of enzymes on flour constituents causing a decrease in the lightness (L) value of flour (Singh *et al.,* 2017a).

Emulsifying properties

Germination time and temperature exert a significant positive effect on the emulsifying properties (emulsion activity (EA) and stability (ES)) of pigeon pea flour (Table 2). Control flour showed the lowest EA (49.73%) and ES (42.62%). An increase in germination time and temperature significantly increased the EA by 6.81 to 34.58% and the ES by 3.99 to 65.36% compared to non-germinated flour. The present study showed that extended germination time and higher temperature resulted in better emulsifying properties (Table 2). During germination, dissociation and fractional unfolding of polypeptide chains occurs, exposing the hydrophobic sites of the amino acids. These are able to form associations with lipid droplets, creating a higher EA (Singh and Sharma, 2017). Denatured proteins – being the food surface active agents – help in creating electrostatic repulsion on the surface of oil droplets in the emulsion, thereby stabilising the emulsion (Singh *et al.,* 2017b). Ghavidel and Prakash (2006) reported that flour from germinated legumes had better emulsification capacities than native flours.

Foaming properties

Foaming capacity (FC) and foaming stability (FS) are important functional properties of flour that are influenced by protein conformation. Both were significantly (P<0.05) and positively enhanced by germination (Table 3). Control flour exhibited the lowest FC of 31.99%, which increased significantly with germination time and temperature. The FC increased from 34.17 to 132.6% as germination time increased from 12 to 48 h and temperature from 25 to 35 °C. During germination, the denaturation of proteins causes a reduced surface tension of air-water molecule inter-phase and an increase in the amount of solubilised proteins. This results in a higher hydrophobic interaction and thus an enhanced FC (Singh and Sharma, 2017). A similar effect on the FC of legumes has been reported by Ghavidel and Prakash (2006).

Germination conditions also improved the FS of the flour (Table 3). The FS of germinated legume flour after 60 min was significantly (P<0.05) increased from 34.07 to 81.55% compared to the control. The enhancement in FS is attributed to surface denaturation of proteins during germination, which reduced the surface tension of air and water molecules. A higher FC of flour makes it suitable for the preparation of gluten-free products and is also useful in improving the textural properties of cakes and confections (Kaushal *et al.,* 2012).

Table 3. Effect of germination conditions on foaming properties of pigeon pea flour.[1,2]

Treatment	Temp. (°C)	Time (h)	Foaming capacity (%)	Foaming stability (%)					
				10 (min)	20 (min)	30 (min)	40 (min)	50 (min)	60 (min)
Control	–	–	31.99±1.08^{i}	87.68±0.22^{e}	78.78±0.88^{g}	66.58±1.33^{f}	53.77±0.88^{g}	44.86±1.55^{h}	29.38±1.77^{g}
Soaked	25	10	37.01±1.25^{h}	81.46±0.57^{g}	76.19±0.76^{h}	65.52±0.96^{fg}	51.61±0.38^{h}	40.93±0.57^{i}	24.86±0.76^{h}
Germination conditions	25	12	43.16±1.46^{gR}	93.25±0.82^{abP}	83.87±0.66^{efQ}	72.52±0.98^{eR}	65.45±0.82^{bcP}	48.88±0.33^{gR}	34.87±0.16^{fR}
		24	51.14±1.73^{efQ}	92.10±0.28^{bcP}	86.53±0.69^{abP}	78.32±0.14^{bP}	64.82±0.41^{cdP}	61.21±0.83^{bP}	41.46±0.55^{eQ}
		36	52.25±1.77^{eQ}	90.05±0.14^{dQ}	86.32±0.27^{abP}	74.83±0.27^{cdeQ}	61.34±0.14^{fQ}	57.70±0.41^{deQ}	42.30±0.27^{eQ}
		48	61.75±2.09^{cdP}	89.39±0.46^{deQ}	84.45±0.34^{cdePQ}	76.28±0.23^{bcPQ}	66.48±0.11^{bcP}	60.40±0.69^{bcP}	47.61±0.92^{dP}
	30	12	52.25±1.77^{eR}	84.40±0.27^{fQ}	75.02±0.54^{hR}	63.25±0.14^{gR}	50.33±0.81^{hR}	40.48±0.41^{iR}	36.84±0.68^{fR}
		24	58.78±1.99^{deQR}	92.21±0.48^{abcP}	85.57±0.72^{bcdP}	73.32±0.24^{deQ}	63.03±0.12^{defQ}	52.99±0.36^{fQ}	37.54±0.63^{fR}
		36	64.01±2.17^{bcPQ}	91.08±0.66^{cdP}	82.96±0.22^{efQ}	73.74±0.44^{deQ}	64.45±0.55^{cdePQ}	57.96±0.22^{cdeP}	43.82±0.11^{eQ}
		48	69.85±2.36^{abP}	92.20±0.81^{abcP}	81.68±0.10^{fQ}	76.31±0.61^{bcP}	66.14±0.40^{bcP}	59.13±0.61^{cdP}	50.32±0.30^{cP}
	35	12	56.06±1.90^{deR}	91.23±0.38^{bcdQ}	84.28±0.63^{defR}	73.67±0.76^{deR}	62.79±0.50^{efR}	51.90±0.25^{fS}	46.46±0.13^{dR}
		24	61.56±2.08^{cdQR}	93.08±0.69^{abcPQ}	84.71±0.34^{cdeR}	74.89±0.23^{cdeQR}	66.93±0.46^{bQ}	55.72±0.69^{eR}	47.27±0.23^{dR}
		36	70.23±2.38^{abPQ}	92.90±0.50^{abcPQ}	86.92±0.10^{bQ}	75.75±0.40^{cdQ}	66.99±0.10^{bQ}	58.52±0.20^{cdQ}	53.11±0.60^{bQ}
		48	74.41±2.52^{aP}	94.20±0.19^{aP}	90.31±0.38^{aP}	84.73±0.10^{aP}	80.97±0.48^{aP}	75.52±0.38^{aP}	62.08±0.38^{aP}

[1] Values having different superscript letters in a column differ significantly (P<0.05) among different treatments.
[2] Values having different capital superscript letters differ significantly (P<0.05) for activation time within each temperature.

Freeze-thaw stability (% synereis)

Freeze-thaw stability indicates the retro-gradation tendency of starch and reflects the quality of the food product. Freeze-thaw stability was significantly influenced by germination conditions (Table 4). Control flour pastes had the lowest syneresis (13.81%). Germination significantly (P<0.05) increased the syneresis of flour from 15.01 to 36.00%. As germination time increased from 12 to 48 h and temperature from 25 to 35 °C, syneresis increased from 43.83 to 138.88% during first freeze-thaw cycle. This increase is due to breakdown and depolymerisation of flour components by enzymes. Depolymerised components are more prone to synereis compared to native flours (Singh and Sharma, 2017). However, the % syneresis significantly decreased (P<0.05) after the second or third freeze-thaw cycle (Table 4). Studies of Elkhalifa and Bernhardt (2013) reported that germination time increased the % syneresis of sorghum flour and an increased number of thaw-cycles tended to decrease % syneresis.

Protein solubility

Protein solubility is the most important functional property of the flour which influences the emulsifying, foaming and gelation properties of flour (Singh and Sharma, 2017). Germination had a significant (P<0.05) positive effect on the protein solubility of flour (Table 4). Control flour had lowest protein solubility (51.46%) which after germination increased significantly.

As germination time increased from 12 to 48 h and temperature from 25 to 35 °C, protein solubility of germinated flour increased from 24.19 to 69.51%. During germination there is a steady and continuous degradation of proteins into shorter peptides and free amino acids by hydrolysis, resulting in enhanced protein solubility of flour (Singh and Sharma, 2017). Protein solubility of green gram and cowpea is also increased by germination (Ghavidel and Prakash, 2006). The higher protein solubility of pigeon pea flour makes it promising for the preparation of novel food products where maximum solubility of proteins is very desirable.

Least gelation concentration

The ability of flours to form gels on heating is an important functional property which occurs when starch and proteins form a 3D network that opposed to flow under pressure. Germination conditions significantly (P<0.05) influenced the LGC of the pigeon pea flour (Table 5). Control flour exhibited an LCG of 8% with firm gel formation. The increase in germination time from 12 to 48 h and temperature from 25 to 35 °C significantly increased the LGC of the flour from 12 to 25%. Changes in the characteristics of proteins, carbohydrates and degradation of flour components by amylases and proteases may have an influence on gel formation. Similar results were reported by Chinma *et al.* (2009) in tingernut flour and by Benítez *et al.* (2013) in non-conventional legume flour. Flour with a high LGC has an advantage in the preparation of weaning foods.

Table 4. Effect of germination conditions on syneresis (%) with repeated freeze-thaw cycles (TC) and protein solubility of pigeon pea flour.[1,2]

Treatment	Temperature (°C)	Time (h)	% Syneresis			Protein solubility (%)
			TC 1	TC 2	TC 3	
Control	–	–	13.81±0.22^{l}	11.54±0.14^{k}	8.41±0.08^{l}	51.46±0.76^{k}
Soaked	25	10	14.07±0.15^{kl}	11.75±0.10^{k}	9.30±0.03^{k}	52.54±0.47^{jk}
Germination conditions	25	12	15.01±0.66^{jkS}	12.76±0.02^{jR}	9.69±0.02^{kS}	54.62±0.83^{jS}
		24	16.56±0.12^{hR}	13.00±0.13^{jR}	10.49±0.00^{jR}	61.96±0.55^{iR}
		36	20.24±0.12^{gQ}	15.35±0.10^{gQ}	15.52±0.00^{fQ}	66.91±0.21^{hQ}
		48	29.71±0.00^{dP}	20.14±0.05^{eP}	19.08±0.19^{eP}	71.86±0.16^{fP}
	30	12	14.70±0.15^{klS}	13.56±0.01^{iS}	10.29±0.00^{iS}	60.08±0.92^{iS}
		24	15.90±0.28^{hiR}	14.87±0.17^{hR}	12.18±0.02^{hR}	69.36±0.57^{gR}
		36	26.80±0.10^{eQ}	24.02±0.02^{dQ}	22.05±0.00^{dQ}	80.31±0.48^{dQ}
		48	33.29±0.33^{bP}	29.71±0.00^{bP}	24.57±0.10^{bP}	84.74±0.18^{cP}
	35	12	15.20±0.27^{ijS}	14.31±0.14^{hS}	11.48±0.07^{iS}	77.04±0.17^{eS}
		24	24.28±0.02^{fR}	18.80±0.00^{fR}	14.46±0.10^{gR}	86.09±0.51^{cR}
		36	31.97±0.10^{cQ}	27.08±0.12^{cQ}	23.72±0.13^{cQ}	91.10±0.29^{Q}
		48	36.00±0.17^{aP}	32.72±0.13^{aP}	27.68±0.08^{aP}	94.70±0.57^{aP}

[1] Values having different superscript letters in a column differ significantly (P<0.05) among different treatments.
[2] Values having different capital superscript letters differ significantly (P<0.05) for activation time within each temperature.

Table 5. Effect of germination conditions on least gelation concentration (LGC) of pigeon pea flour.[1]

Treatment	Temperature (°C)	Time (h)	Flour concentration% (W/V)											
			2	4	6	8	10	12	14	16	18	20	25	30
Control	–	–	–	–	±	+	+	+	+	+	+	+	+	+
Soaked	25	10	–	–	±	+	+	+	+	+	+	+	+	+
Germination conditions	25	12	–	–	±	±	+	+	+	+	+	+	+	+
		24	–	–	–	±	+	+	+	+	+	+	+	+
		36	–	–	–	±	+	+	+	+	+	+	+	+
		48	–	–	–	±	±	+	+	+	+	+	+	+
	30	12	–	–	–	±	+	+	+	+	+	+	+	+
		24	–	–	–	–	±	±	+	+	+	+	+	+
		36	–	–	–	–	±	±	±	+	+	+	+	+
		48	–	–	–	–	–	–	±	±	+	+	+	+
	35	12	–	–	–	–	–	±	±	+	+	+	+	+
		24	–	–	–	–	–	–	±	±	+	+	+	+
		36	–	–	–	–	–	–	±	±	±	+	+	+
		48	–	–	–	–	–	–	–	–	±	±	+	+

[1] – = not gelled; ± = gelled slightly; + = gelled completely.

4. Conclusions

The results from the present study show that germination of pigeon pea can be employed as a natural processing technique to modify the functional characteristics and protein solubility of pigeon pea flour. Germination increases the enzymatic activity in the grains which results in modification of proteins, starch and other polysaccharides, and can enhance many desired functional properties of pigeon pea flour for food applications. The results

suggest that flour prepared from germinated grains can be efficiently utilised in a variety of novel food products. Natural modified flour from pigeon pea can have great potential in the preparation of gluten free food, weaning food for infants and functional foods due to their better nutrient digestibility.

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