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Research article

Physicochemical, functional and rheological properties of grass pea (*Lathyrus sativus* L.) flour as influenced by particle size

Manju Bala [a,*], Saksham Handa [b], Mridula D [a], R.K. Singh [a]

[a] *ICAR-Central Institute of Post-Harvest Engineering and Technology, Ludhiana, Punjab, India*
[b] *Thapar Institute of Engineering and Technology, Patiala, Punjab, India*

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ABSTRACT

Different properties of grass pea (*Lathyrus sativus* L.) flour passed through 60, 72, 85, 100, 150 and 200 mesh sieves and in the size range of 249-74 μm were studied. The protein content reduced while fat content improved significantly with diminution in particle size. Flowability, capacities to absorb water and form foam as well as stability of foam decreased while increase in swelling capacity, swelling index, oil absorption capacity was observed with decrease in flour particle size. Bimodal curve patterns were obtained for coarse flour samples of 249 μm and 211 μm using light scattering analysis. Scanning electron microscopy analysis revealed that starch granules were of variable shape embedded in protein and fibre matrix. The flour with fine particle size exhibited greater values for peak, final, break down and set back viscosities and the dough from fine particles showed highest values for storage modulus (G′), loss modulus (G″),|G*| and |η*|.

1. Introduction

Grass pea (*Lathyrus sativus* L.) is commonly known as *khesari* dal is one of the traditional pulses in India. India produced 287 thousand tons of grass peas from 357 thousand hectare area during 2017–18 (India Agristat, 2019). Because of its use as a food, feed, fodder and green manure it is considered as a potential crop. The seeds of grass pea comprise of carbohydrates, proteins, fat and ash content of 58%, 31%, 2% and 2%, respectively expressed on dry weight basis (Aletor et al., 1994; Akalu et al., 1998). It contains good quantities of essential amino acids except the sulfur containing amino acids. Besides proteins, grass pea seeds also contain magnesium, phosphorus and calcium like minerals (Urga et al., 2005).

Although it is considered as a potential crop, but presence of a toxic, and unusual amino acid known as β-N-oxalyl-L-α, β-diaminopropionic acid (ODAP) or β-oxalyl amino alanine (BOAA), has lowered its applications. There are reports that excessive intake of the seeds/dal for prolonged time might cause neurolathyrism (Jiao et al., 2011). As per Lambein et al. (2019) the extremely good agronomic and nutritional qualities of this plant have been overlooked till date due to the negative publicity of the supposed toxic effects. Efforts are being done by the researchers in recovering the diffusion of this crop worldwide (Romano et al., 2019). In this context, India has released some low toxin varieties *viz.*, Ratan, Prateek and Mahateora with ODAP content of 0.07–0.10% for general cultivation (Kumar et al., 2011).

Recently consumers are showing more interest in legume proteins and industries are looking for the unconventional sources of proteins with distinctive functionalities (Romano et al., 2019). This crop can grow under drought conditions and is a cheap and rich source of proteins. In this sense, it can serve as a cheap protein source for industrial use in the form of flour.

Flour properties have been reported to be influenced by its constituent composition as well as the surface properties of particles. Size reduction of grains is a key unit operation for increasing the ratio of surface area to volume of resulting flours. Milling and grinding produces flour samples having heterogeneous mixture of varied particles. The inherent and functional characteristics of flour are strongly affected by its particle size, which in turn are important for the quality of product derived from that flour. Particle size distribution provides full description of a flour or powder. Sieving of flour can produce sample with uniform particle size distribution resulting in the development of product with better functional properties. Relation of particle size and different properties of legume flour of cowpea (Kerr et al., 2000), lentil (Ahmed et al., 2016), black gram (Sun et al., 2019) etc. has been reported but as far as our knowledge is concerned till date no report on inherent properties of grass pea flour showing their dependence on the

* Corresponding author.
E-mail address: avimithi@gmail.com (M. Bala).

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flour particle size is available. Considering that grass pea is gaining importance as a cheap source of proteins and could find applications in the development of newer products, present work has been designed to find out the effects of particle size on proximate analysis, physico-chemical, functional and rheological characteristics of lesser known and underutilized grass pea flour.

2. Materials and methods

2.1. Materials

Grass pea (*Lathyrus sativus* L.) seeds of variety Mahateora were procured from Deptt of Agri Process and Food Engg., Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh (India).

2.2. Preparation of flour

Seeds were processed by dehusking and milling to prepare grass pea dal using AKOLA dal mill (Maa Durga Agencies, Maharashtra, India) at ICAR-CIPHET, Ludhiana, Punjab, India. Dal was ground in flour mill having a sieve with 0.5 mm pore size (Natraj, Scorpio Entreprises, Ahmedabad, India). Further, different grass pea flour fractions were prepared by sieving the flour through a series of British sieve size (BSS) 60, 72, 85,100,150, 200 mesh sieves. After sieve analysis, the fractions which were retained on the sieve were assigned as 249 (-60; +72), 211 (-72; +85), 179 (-85; +100), 149 (-100; +150), 105 (-150; +200), and 74 (-200; +pan) μm. The –ve size represents grass pea flour passed through the sieve and retained particles are expressed using + sign (Ahmed et al., 2014). Samples were filled in air tight containers for storage in refrigerator at 4 °C.

2.3. Proximate composition and β-ODAP estimation of flour

Proximate compositon of samples was evaluated for proximate components *viz.* moisture, protein, fat and ash contents in triplicates as per the methods described by AOAC (2000). Total carbohydrate content was computed by difference after determination of moisture, protein, fat and ash content. The content of β-ODAP was estimated as per method of Rao (1978). For this purpose 25 mg of the flour sample was added to 5 ml of distilled water, and the mixture was kept at 100 °C in a water bath for 30 min. Centrifugation of the mixture was carried out at 4000 rpm (10 min). 0.9 ml of distilled water was added to the 0.1 ml of the supernatant. In each tube 0.2 ml of KOH (3 N) was added. Each sample was taken in duplicate, contents of one set of the tubes were boiled for 30 min other set was maintained at room temperature (RT). The tubes were cooled at room temperature and 2 ml of 0.5 M Dipotassium tetraborate buffer (pH 9.9) was added, followed by the addition of 2 ml of o-phthalaldehyde (OPT reagent). OPT reagent was prepared by mixing o-phthalaldehyde (100 mg), 95% ethanol (1 mL) and 0.5 M dipotassium tetraborate buffer of pH 9.9 (99 ml) and 0.2 ml of mercaptoethanol was added to it. After 30 min the optical density was measured at 420 nm. DAP was estimated from the difference in optical density of hydrolysed and unhydrolysed sample. Standard curve was prepared using different concentrations of standard DAP (100 μg/ml) and the ODAP content was calculated in accordance with the following equation (Eq. (1)):

$$\text{ODAP in sample } (\%) = 1.25 \times \text{DAP } (\%) \tag{1}$$

2.4. Flowability

2.4.1. Bulk density and tapped density

Bulk density (ρ_b) of the samples was ascertained by filling flour sample in a container of known volume (V_0) (pre weighed 10 mL graduated cylinder with least count of 0.5 mL). Both weight and volume of the contents were noted. Bulk density was expressed as kg/m^3 and calculated as given in Eq. (2):

$$\text{Bulk Density} = M/V_0 \tag{2}$$

The tapped volume (V_f) was calculated from Eq. (3) after tapping the measuring cylinder having known amount of sample, twenty times on a flat table top surface.

$$\text{Tapped density (kg/m}^3) = M/V_f \tag{3}$$

2.4.2. Carr Index and Hausner ratio

Carr compressibility index (CI) and Hausner ratio (HR) were computed as per method of Carr (1965) and Hausner (1967) using following formulae (Eqs. (4) and (5)):

$$CI = \frac{\rho tap - \rho bulk}{\rho tap} * 100 \tag{4}$$

$$HR = \frac{\rho tap}{\rho bulk} \tag{5}$$

Where ρtap is the tap density and ρbulk represents the bulk density of flour sample.

2.5. Color measurement and pH of the flour

Color characteristics of the flour fractions were analyzed with hand held colorimeter (Hunter Lab mini Scan XE Plus colorimeter; Model 45/0-L, HAL, USA) on the basis of L, a and b values. The calibration of the colorimeter was done using the color standards. The results were then recorded using D-65 illuminant at a viewing angle of 10°. Triplicate measurements were performed for each sample. The values of chroma (C) and hue angle (h^o) were calculated from the Eqs. (6) and (7) (Little, 1975):

$$C = \sqrt{(a)^2 + (b)^2} \tag{6}$$

$$h\,(^\circ) = arc\,\tan\left(\frac{b}{a}\right) \tag{7}$$

The pH of the grass pea flour samples was estimated (Suntharalingam and Ravindran, 1993) using 4 g flour in 100 ml water and agitated thoroughly. After 30 min supernatant was decanted and pH was measured.

2.6. Particle size distribution

Particle size analyzer (Horiba Partica LA-950V2) in the wet dispersion mode using methanol and ultrasonication was used to measure particle size data of the flour fractions. Particle sizes at 10% (D_v10), 50% (D_v50), median diameter and 90% (D_v90) of the volume distribution were computed. The specific surface area (m^2/m^3) was also expressed.

2.7. Scanning electron microscopy (SEM)

Scanning Electron microscope (Model No. JSM-5510; JEOL Ltd., Tokyo, Japan) was used to study the microstructures of the flour samples. Samples were prepared by coating with gold in a sputter coater (JEOL JFC-1600 Auto fine coater). Samples were attached on aluminium stubs (32 mm) using a double backed cellophane tape. SEM was operated at 15 kV voltage, with 32 mm holder size and 11 mm working distance.

2.8. Water absorption capacity (WAC), water solubility index (WSI) and oil absorption capacity (OAC)

The Water absorption capacity (WAC) and water solubility index (WSI) was determined by the method of Kadan et al. (2008). Flour

sample (2 g) was taken in pre-weighed centrifuge tube and to it 20 ml of distilled water was addded. The suspension was kept for 2 h at room temperature with occasional stirring and then centrifuged at 3000 rpm for 10 min. The supernatant was carefully poured into pre-weighed petridish and kept for the estimation of water solubility index (WSI). Weight of the wet sediment was taken. The WAC was calculated as below (Eq. (8)):

$$\text{WAC (g / g)} = \frac{\text{Weight of wet sediment (g)}}{\text{Dry weight of flour (g)}} \quad (8)$$

The supernatant obtained in the estimation of WAC was dried at 105 °C for overnight and weighed. The water solubility index (WSI) was calculated as Eq. (9):

$$\text{WSI (\%)} = \frac{\text{Weight of dried supernatant (g)}}{\text{Dry weight of flour (g)}} \times 100 \quad (9)$$

For estimation of oil absorption capacity (OAC) method of Olu et al., 2012 was used. 2.5 g of sample was mixed with 25 ml of groundnut oil in a pre-weighed centrifuge tube. The mixture was stirred for 1 min followed by centrifugation at 4000 rpm (20 min). After that, the extra oil was decanted and contents of the tube were weighed. OAC was calculated as follows Eq. (10):

$$\text{OAC (g / g)} = \frac{\text{Weight of oil absorbed (g)}}{\text{Weight of sample (g)}} \quad (10)$$

2.9. Swelling index and swelling capacity

Swelling Index (SI) of the flour samples was determined by the method of Ukpabi and Ndimele (1990). The sample was added up to 10 ml mark in a pre-weighed 100 ml measuring cylinder. The cylinder was again weighed in order to obtain the sample weight. Distilled water was added up to the 50 ml mark and mixed properly using vortex mixer to homogenize the sample. The mixture was allowed to stand for 3 h. The swelling index was calculated as Eq. (11):

$$\text{SI} = \frac{\text{Volume of sample after soaking} - \text{Volume of sample before soaking}}{\text{Weight of sample}} \quad (11)$$

The wet sediment obtained from swelling index determination was used in calculating swelling capacity (SC) using following formula (Eq. (12)):

$$\text{SC (\%)} = \frac{\text{Weight of wet sediment}}{\text{Weight of sample}} \times 100 \quad (12)$$

2.10. Foaming capacity and foaming stability

Foaming capacity (FC) and Foaming Stability (FS) was determined by the method of Kaur and Singh, 2005 with some modifications. 1 g of flour sample was taken in a 250 ml beaker and to it 50 ml of distilled water was added. The contents were stirred for 1 min using home blender. Immediately the contents were transferred to a 100 ml graduated measuring cylinder. The final volume of the foam (ml) was noted and the foaming capacity of flour was calculated as Eq. (13):

$$\text{FC (\%)} = \frac{(\text{Vol. of foam after whipping} - \text{Vol. of foam before whipping})}{\text{Vol. of foam before whipping}} \times 100 \quad (13)$$

Foaming stability (FS) was determined by measuring the decrease in the volume of foam (ml), after every 10 min for 1 h. It was calculated using the formula (Eq. (14)):

$$\text{FS (\%)} = \frac{\text{Volume of foam after 1 hour}}{\text{Initial foam volume}} \times 100 \quad (14)$$

2.11. Pasting properties

Pasting properties of grass pea flour samples were determined using a rapid visco analyzer (RVA) (Newport Scientific model 4-SA, Warriewood, Austria) by following AACC (2000) method. Flour suspensions were prepared by dispersing 3 g flour sample in 25 ml water. The pasting time, temperature, peak viscosity (PV), trough or hot paste viscosity (HPV), final or cold paste viscosity (FV or CPV), setback (SV = CPV-HPV) and breakdown (BD = PV-HPV) viscosity parameters were obtained directly from software.

2.12. Rheological characterization of dough

Rheological characterization of flour dough samples of different particle size was carried out with Rheometer (MCR 301, Anton Paar GmbH, Germany), using parallel plate probe of 25 mm diameter (PP25). All the measurements were accomplished in duplicate at a temperature of 25 °C. A fixed gap of 1 mm was set between plates. Linear viscoelastic region (LVE) was determined for different dough (prepared with same water content, using flour/water ratio (w/v) of 1:1) at a constant frequency of 1 Hz and strain of 0.01–100%. Different types of dough were characterized for storage modulus (G′), loss modulus (G″), and |G*| and complex viscosity (|η*|) using the frequency sweep tests within the LVE at a constant deformation (0.05% strain) and the frequency range of 0.1–100 Hz.

2.13. Statistical analysis

All measurements and analysis were done in three replications, shown as mean ± standard deviations. The least significant differences among the mean values of studied parameters of different particle size were examined using one-way analysis of variance (ANOVA) and the Tukey's Multiple Comparison Test ($p < 0.05$).

3. Results and discussion

3.1. Proximate composition and β-ODAP content

The results of proximate composition of grass pea flour samples as dependent on the particle size are presented in Table 1. The results revealed that the reduction in particle size caused significant changes in constituents of the flour samples. The moisture content values ranged from 8.60 to 9.80% and did not differ significantly with reduction in particle size, except for flour with particle size 179 μm which showed lowest moisture content. The protein content of grass pea flour samples decreased slightly as the flour particle size decreased from 249 μm-105 μm and with further reduction to 74 μm size a significant ($p < 0.05$) drop was noticed. The flour sample with particle size 249 μm and 74 μm showed the highest (28.36%), and the lowest (21.96%) protein content, respectively. Comparable decline in protein content with decreasing particle size for rice flour samples have been reported Kim and Shin (2014) and Ahmed et al. (2015). In contrast to our findings, Sullivan et al. (1960) observed a rise in the protein content values when the particle size was reduced in the case of hard wheat flour. They reported that the variation in the protein content with particle size is different for different crops, due to variation in the structure and morphology of endosperm.

Fat content of grass pea flour increased significantly with lowering in particle size. Flour sample with particle size of 149 μm, exhibited an elevated fat content of 2.64% which further decreased significantly to

Table 1. Proximate analysis of grass pea flour of different particle sizes.

Particle size (μm)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Total carbohydrate (%)	Gross energy value (Kcal/100 g)	β-ODAP content (mg/100 g)
249	8.78 ± 0.75^{a}	28.36 ± 2.52^{a}	1.26 ± 0.07^{e}	2.83 ± 0.20^{a}	58.76 ± 3.05^{a}	359.9 ± 2.90^{a}	12 ± 0.00^{a}
211	9.12 ± 0.23^{a}	28.51 ± 1.83^{a}	1.48 ± 0.10^{de}	2.90 ± 0.10^{a}	57.98 ± 1.82^{a}	359.3 ± 1.08^{a}	14 ± 0.00^{a}
179	8.60 ± 0.14^{b}	27.82 ± 1.61^{a}	1.78 ± 0.07^{cd}	2.73 ± 0.37^{a}	59.06 ± 1.78^{a}	363.6 ± 1.39^{a}	14 ± 0.00^{a}
149	9.41 ± 0.32^{a}	27.05 ± 1.89^{a}	2.64 ± 0.29^{a}	2.93 ± 0.11^{a}	57.96 ± 2.06^{a}	363.9 ± 2.66^{a}	07 ± 0.00^{b}
105	9.40 ± 0.38^{a}	26.80 ± 1.05^{a}	2.08 ± 0.02^{bc}	2.93 ± 0.32^{a}	58.78 ± 0.76^{a}	361.0 ± 1.48^{a}	07 ± 0.00^{b}
74	9.80 ± 0.43^{a}	21.96 ± 0.44^{b}	2.23 ± 0.07^{b}	3.20 ± 0.10^{a}	62.80 ± 0.73^{a}	359.1 ± 1.40^{a}	07 ± 0.00^{b}

Results are expressed as mean values ± standard deviations of three replications. Means in a column with same superscripts are not significantly different ($p \leq 0.05$).

2.23% on reduction in particle size. Ahmed et al., 2015 observed the highest levels of fat in the finest (74 μm) rice flour and related it to the availability of more surface area to the solvent during fat extraction. Grass pea flour samples revealed the ash content in the extent of 2.73–3.20% which did not differ significantly with change in particle size. Our results are in concordance with those of Kim and Shin (2014), Ahmed et al. (2019) who did not find any significant differences in ash contents with reduction in particle size of rice, and quinoa flour samples, respectively. However, Ahmed et al. (2016) reported a drop in ash content for lentil flour as the particle size reduced from 210 to 75 μm in linear fashion.

Total carbohydrate content (57.96–62.80 %) and the gross energy value of the flour samples showed variable values differing in a non-significant manner for the flour samples with decreasing particle size. The value of protein, fat, ash, and moisture in grass pea flour fractions are in unison with the already reported data of Carbonaro et al., 2015 and Romano et al., 2019. The data showed that flour with particle size of 249 μm showed high protein content, low fat, ash and carbohydrate content while flour fraction of 74 μm particle size showed minimum protein content with high fat, ash and carbohydrate content. These differences could be due to the cellular distribution of these components in grass pea sample, which are further differentially distributed by grinding and sieving.

The results revealed that the β-ODAP content (Table 1) ranged from 7 mg/100 g to 14 mg/100 g for different flour fractions. The significant decrease in the β-ODAP content was remarked when the flour particle size was narrowed from 179 μm to 74 μm. β-ODAP content in the range of 20–700 mg/100 g on dry matter basis has been reported by Deshpande and Campbell, 1992 from grass pea seeds of 100 accessions from various countries. Hanbury et al. (2000) compiled data of β-ODAP content in raw grass pea from various countries (mg/100 g) as 160 to 250 (Spain), 70 to 750 (Syria), 40 to 760 (Australia), 450 to 1400 (Bangladesh), 280 to 1500 (India), 180 to 520 (Chile), and 80 to 990 (China) and very high levels have been reported. However, the variety under study has been reported to contain 70–100 mg/100 g (Kumar et al., 2011). But our results showed very low levels of β-ODAP content in grass pea flour samples of different particle size. This fall in β-ODAP content could be ascribed to processing of grass pea seeds to flour and further size fractionation by sieving. The study reveals that particle size fractionation could be used as a means to minimize the level of β-ODAP content in the flour samples.

3.2. Flour flowability

3.2.1. Bulk density and tapped density

Bulk density determines the flour expansion and porosity of products. The bulk densities of grass pea flour samples of different particle sizes decreased from 460 kg/m^3 to 420 kg/m^3 as the flour particle size decreased from 249 μm to 74 μm (Table 2). This decrease in bulk density was significant from 249 μm to 211 μm and became insignificant with further reduction in particle size. However, Sun et al. (2019) had reported a decline in bulk density from 0.439 g/mL to 0.364 g/mL as the flour particle size reduced from 250–180 μm to <38 μm for black kidney bean powder. Ahmed et al. (2015) found no significant changes in the bulk density of the rice flour samples with change in particle size, and reported that the significant decrease occurred only for finest particle size flour (74 μm). It has been stated that the increased values of bulk density are contributed by the flour fractions having larger particle size. The decrease in bulk density in finer flour fraction could be due to reduced protein content and slightly higher ash content (Ahmed et al., 2015).

Similar to bulk density, the tapped density also decreased from 525 kg/m^3 to 520 kg/m^3 with lowering in the particle size of flour samples from 249 μm to 74 μm and this decrease was not significant. It was observed that the values for tapped density for various samples were more than their respective values for bulk density. It has been observed that the free flowing powders with coarser particles had lower compaction characteristics while the fine powders settled rapidly due to tapping (Abdullah and Geldart, 1999). The flour fraction with increased bulk density is acceptable for lessening the thickness of paste by virtue of its reduced viscosity and find applications in food stuffs that possess coarse texture. Flour with less bulk density could be used to devise the supplementary foods with even and packed texture.

3.2.2. Carr Index and Hausner ratio

The Carr compressibility Index and Hausner ratio are measures of the product ability to settle and also provide information about flow behavior of the flour samples. Carr Index value of 12 increased to 19.33, with decrement in particle size from 249 μm to 149 μm (Table 2). Further, the decrease in size did not affect these values. Hausner ratio

Table 2. Effect of particle size flow properties of grass pea flour.

Particle size (μm)	Bulk density (kgm^{-3})	Tapped density (kgm^{-3})	Carr Index (%)	Hausner Ratio
249	460 ± 0.00^{a}	525 ± 0.01^{a}	12.00 ± 2.00^{c}	1.13 ± 0.02^{c}
211	415 ± 0.00^{b}	486 ± 0.00^{a}	14.67 ± 3.05^{bc}	1.17 ± 0.04^{bc}
179	420 ± 0.00^{b}	500 ± 0.01^{a}	16.00 ± 2.00^{bc}	1.19 ± 0.03^{bc}
149	412 ± 0.01^{b}	522 ± 0.01^{a}	19.33 ± 1.15^{ab}	1.24 ± 0.01^{ab}
105	418 ± 0.01^{b}	519 ± 0.01^{a}	19.33 ± 1.15^{ab}	1.24 ± 0.01^{ab}
74	420 ± 0.01^{b}	520 ± 0.02^{a}	19.33 ± 1.15^{ab}	1.24 ± 0.01^{ab}

Results are expressed as mean values ± standard deviations of three replications. Means in a column with same superscripts are not significantly different ($p \leq 0.05$).

ranged from 1.13-1.24 and showed the same trend as was observed for Carr Index. CI values of 11–15, and 16–20, while HR values of 1.12–1.18, and 1.19–1.25, respectively, refers to good and fair flowability (Carr, 1965). The results showed that flour sample with particle size 249 μm and 211 μm had good flowability while all other samples were having fair flowability. Inverse relation of CI & HR with flowability has been reported. Poor flowability of the soybean powder of 250–150 μm particle size (Lee and Yoon, 2015) and very poor flow behavior for unripe banana flour with all studied particle size of <212 to 501-700 μm (Savlak et al., 2016) has been reported. The increased surface area per unit mass of the powders with the small particle size can be associated with their poor flowability. Increased cohesive and frictional forces are operational when the contact surface area between the powder particles is more, which in turn decreases the flow of powder. The higher values of CI, and HR indicated the cohesive nature of the flours, which may cause the difficulties of improper flows, bridging and discharge problems in hoppers.

3.3. Colorimeter and pH

Table 3 represents the color characteristics of the grass pea flour samples of distinct particle sizes. It was observed that the lightness (L) rised with reduction in particle size and fine flour with particle size 74 μm showed the highest L value of 88.85. Similar results showing increase in L value have been reported for the rice flour and black kidney bean powder samples by Kim and Shin, 2014; Ahmed et al., 2015 and Sun et al., 2019, respectively. This increase in L values has been linked with increase in surface area of fine samples as a result of which more reflection of light takes place. The yellowness (+b) value increased from 19.74 to 20.17 but did not differ significantly. The greenness (–a) values decreased significantly ($p < 0.05$), but in a nonlinear pattern with the decrease in particle size. The drop in color, a and b values of finer fractions may be attributed to the reduction in the pigments due to processing operations. The chroma values enhanced non-remarkably with drop in particle size, whereas the hue angle (^{o}) values elevated exceptionally with decrease in particle size. Chroma (C) and hue angle (H°) are used in providing information on spatial distribution of colors when compared to direct values of tri-stimulus measurements. Chroma values of flour fractions showed a narrow range which indicated that color differences were not large. Moreover, decrease in Chroma or C value of food indicates sample becomes darker.

The data showed that the pH of grass pea flour suspensions of different particle size decreased from 6.30 to 6.21 in a significant but nonlinear manner ($p < 0.05$) with the decrease in particle size (Table 3). However, pH values of unripe banana flour were not affected by the change in particle size (Savlak et al., 2016). Further, pH equal to 6.4 has been reported for grass pea flour (Romano et al., 2019), which was significantly higher than the pH values of other legumes, and was attributed to the lesser amount of tannins, and other acidic components. On the contrary, in the current investigation, the observed decline in the pH of flour fractions, with reduction in flour particle size can be related to the increase in the acidic components.

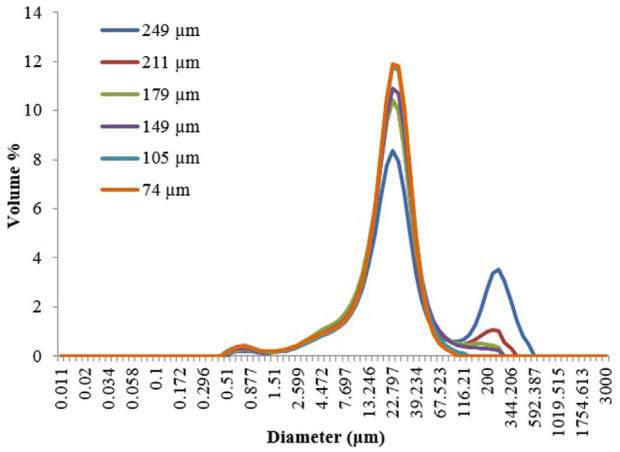

Figure 1. Particle size distribution patterns of grass pea flour samples of different particle sizes.

3.4. Particle size distribution

The particle size distribution (PSD) patterns of grass pea flour samples are shown in Figure 1. Samples in the particle size range of 249 μm and 211 μm, displayed bimodal particle size distribution. As the particle size lowered, peak corresponding to larger particle almost disappeared and only one peak could be seen (Figure 1). The curve patterns of PSD of grass pea flour set forth a greater peak ranging from 1.50 to 100 μm and small peak from 110 to 590 μm. Particle sizes at 10% (D_{10}), 50% (D_{50}) and 90% (D_{90}) volume distribution along with specific surface area were recorded (Table 4). Our results showed that as the particle size diminished from 249 μm and 211 μm, D_{90}, D_{50} and D_{10} values decreased significantly and further reduction in particle size did not cause any significant changes in the particle size distribution while increase in surface area was significant in non-linear way. The average particle size of the flour fractions spanned from 20.61 to 23.71 μm, showing that the flour was finely ground and sieve used at the time of grinding also affects particle size distribution of the flour samples. Hareland, 1994 reported that the different types of particle size distributions for different flours may depend on firmness and grade of material, grinding equipment used and the time required for grinding etc. Impact forces that operate between a steel roll and inner wall of the grinder, during the process of grinding, aids in the process of particle size reduction. From the results it can be interpreted that particle size distribution is affected both by the grinding as well as sieving.

3.5. Scanning electron microscopy analysis

The scanning electron micrographs of grass pea flour at 500× magnification are shown in Figure 2 a-f. The starch particles displayed varying morphology *viz.* round and oval, rectangular and ellipsoidal, were darker in color. They were attached with and also surrounded by white colored protein

Table 3. Effect of particle size on color profile and pH of grass pea flour.

Particle size (μm)	L	a	b	Hue (^{o})	Chroma	pH
249	87.51 ± 0.20^{b}	-0.44 ± 0.10^{a}	19.74 ± 0.30^{a}	-88.71 ± 0.30^{d}	19.74 ± 0.30^{a}	6.30 ± 0.01^{a}
211	88.28 ± 0.14^{a}	-1.00 ± 0.09^{b}	19.76 ± 0.13^{a}	-87.08 ± 0.25^{bc}	19.78 ± 0.13^{a}	6.25 ± 0.02^{ab}
179	88.35 ± 0.20^{a}	-1.03 ± 0.01^{b}	19.86 ± 0.15^{a}	-87.02 ± 0.06^{bc}	19.87 ± 0.15^{a}	6.27 ± 0.02^{ab}
149	88.75 ± 0.20^{a}	-1.14 ± 0.07^{b}	20.17 ± 0.14^{a}	-86.74 ± 0.19^{ab}	20.2 ± 0.14^{a}	6.25 ± 0.00^{ab}
105	88.44 ± 0.27^{a}	-1.27 ± 0.04^{c}	20.10 ± 0.06^{a}	-86.37 ± 0.14^{a}	20.14 ± 0.06^{a}	6.22 ± 0.01^{c}
74	88.85 ± 0.47^{a}	-0.93 ± 0.07^{b}	19.58 ± 0.62^{a}	-87.28 ± 0.12^{c}	19.60 ± 0.62^{a}	6.21 ± 0.00^{c}

Results are expressed as mean values ± standard deviations of three replications. Means in a column with same superscripts are not significantly different ($p \leq 0.05$).

Table 4. Particle size distribution of grass pea flour.

Particle size (μm)	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)	Specific surface area (cm^2/cm^3)
249	7.96 ± 0.15^{a}	23.71 ± 0.26^{a}	235.7 ± 9.80^{a}	4221 ± 81.1^{c}
211	7.06 ± 0.15^{bc}	20.81 ± 0.29^{b}	50.16 ± 4.16^{b}	5289 ± 03.7^{b}
179	6.61 ± 0.07^{c}	20.28 ± 0.17^{b}	42.86 ± 1.29^{b}	5380 ± 11.5^{b}
149	7.06 ± 0.08^{bc}	20.82 ± 0.14^{b}	41.78 ± 0.17^{b}	5433 ± 20.2^{b}
105	7.22 ± 0.08^{b}	20.65 ± 0.09^{b}	36.80 ± 0.03^{b}	5829 ± 03.7^{a}
74	7.00 ± 0.23^{b}	20.69 ± 0.26^{b}	35.72 ± 0.43^{b}	5977 ± 18.3^{a}

Results are expressed as mean values ± standard deviations of three replications. Means in a column with same superscripts are not significantly different ($p \leq 0.05$).

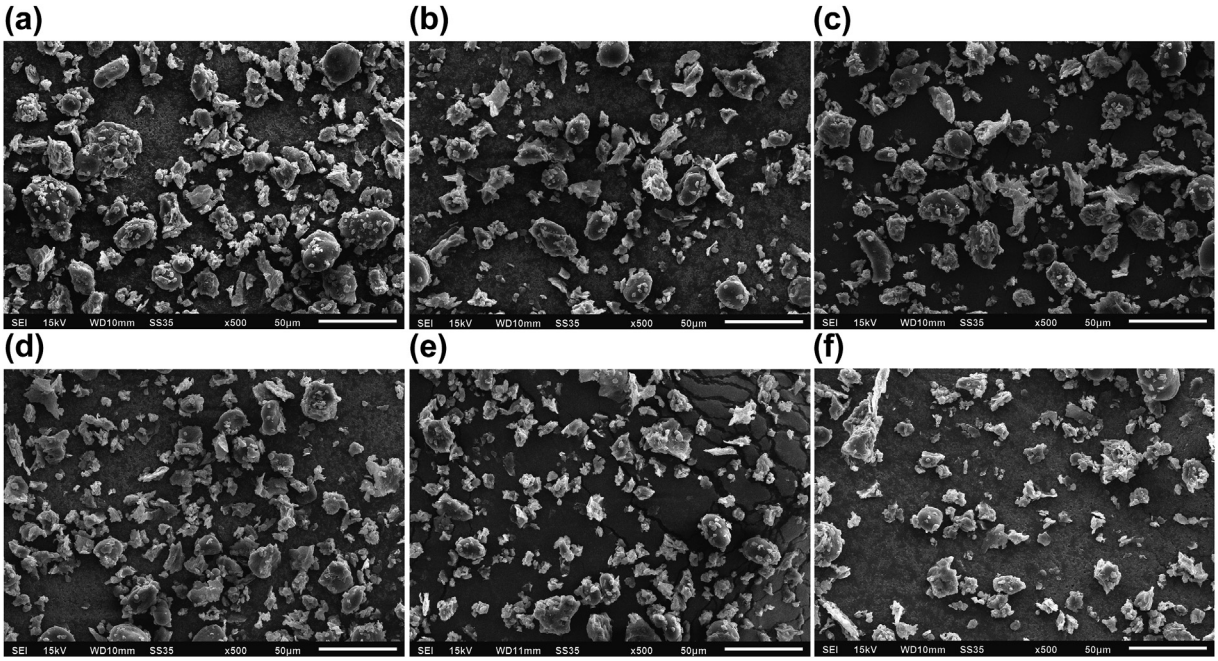

Figure 2. Scanning electron micrographs of grass pea flour samples of different sizes (a) 249 μm (b) 211 μm (c) 179 μm (d) 149 μm (e) 105 μm (f) 74 μm.

entities or remains of protein matrix. Elongated fiber particle were also noticeable. With decrement in particle size, the size of starch granules decreased and tended to be circular in shape in the finest particle size flour. Our results are in line with the results of Romano et al., 2019 who inferred that SEM of grass pea flour (425 μm) revealed the presence of ovoid to ellipsoidal shaped starch granules and globular or irregular particles of protein moieties joined to or present in between the starch particles, along with mineral and fiber components.

3.6. Water absorption capacity (WAC), water solubility index (WSI) and oil absorption capacity (OAC)

Water absorption capacity (WAC) refers to the extent with which water can be bound per gram of flour and is vital for established product qualities such as the ability to retain moisture, starch retrogradation and staling. The WAC varied from 1.75 to 2.12 g/g for grass pea flour samples with different particle sizes (Table 5). WAC was highest (2.12 g/g) and lowest (1.75 g/g) for grass pea flour fraction of 211 μm and 74 μm, respectively and decreased non-significantly with reduction in particle size. However, a significant drop in the WAC with change in size from 88

Table 5. Effect of particle size on WAC, WSI, OAC, SC and SI of grass pea flour.

Particle size (μm)	WAC (g/g)	WSI (%)	OAC (g/g)	SC (%)	SI	FC (%)	FS (%)
249	1.93 ± 0.00^{a}	14.1 ± 4.03^{ab}	0.88 ± 0.00^{d}	211.1 ± 1.42^{b}	0.23 ± 0.01^{c}	70.89 ± 2.34^{b}	75.04 ± 2.41^{ab}
211	2.12 ± 0.24^{a}	13.1 ± 1.09^{ab}	1.10 ± 0.00^{c}	212.6 ± 13.3^{b}	0.27 ± 0.08^{bc}	80.77 ± 2.71^{a}	83.52 ± 2.68^{a}
179	1.89 ± 0.02^{a}	9.70 ± 0.56^{ab}	1.07 ± 0.02^{bc}	212.9 ± 1.15^{b}	0.32 ± 0.00^{abc}	81.73 ± 1.35^{a}	82.84 ± 1.18^{ab}
149	1.79 ± 0.00^{a}	17.6 ± 3.14^{ab}	1.19 ± 0.00^{a}	214.8 ± 13.6^{b}	0.39 ± 0.02^{ab}	60.58 ± 1.35^{c}	79.76 ± 1.13^{a}
105	1.80 ± 0.02^{a}	15.7 ± 1.45^{ab}	1.12 ± 0.02^{bc}	227 ± 8.35^{a}	0.43 ± 0.01^{a}	56.32 ± 0.77^{c}	72.22 ± 3.93^{bc}
74	1.75 ± 0.00^{a}	19.5 ± 2.65^{a}	1.16 ± 0.00^{a}	226.1 ± 4.45^{a}	0.44 ± 0.00^{a}	61.92 ± 2.17^{c}	71.80 ± 3.62^{c}

WHC: Water Holding Capacity, WSI: Water Solubility Index, OAC: Oil Absorption Capacity, SC: Swelling Capacity, SI: Swelling Index, FC: Foaming Capacity, FS: Foaming Stability. Results are expressed as mean values ± standard deviations of three replications. Means in a column with same superscripts are not significantly different ($p \leq 0.05$).

to 74 μm has been observed by Ahmed et al. (2016) in the case of water chestnut flour. The flour showing higher WAC may possess hydrophilic constituents with free groups of carbohydrates and proteins to interact with water.

Water solubility index (WSI), depicts the release of soluble portions of the flour, and ranged from 9.7 to 19.58% (Table 5). It first decreased significantly with decrement in flour size from 249 μm to 179 μm and then improved with further decrease in particle size. Water solubility index is associated with the existence of dispersible molecules like albumins, amylose, sugars, oligosaccharides and other soluble components. The finest particles exhibited the highest WSI, due to more leaching. Similar to our results, Ahmed et al. (2016) also revealed an increase in the WSI values from 11.09 to 13.11% with decrease in particle size for water chestnut flour. Oil absorption occurs due to the physical interactions of oil with hydrophobic groups of proteins. The OAC increased significantly ($p < 0.05$) with drop in particle size from 249 μm to 149 μm (Table 5). Our results are in line with those of Ahmed et al. (2016) who described an increase in OAC of lentil flour fractions from 210 to 105 μm sizes, and further reduction in particle size caused decrease in OAC The lowering of the OAC may be credited to the lower protein content and or less accessibility of hydrophobic chains in the finer flour fractions (Bolade et al., 2009), as it has been proposed that oil may get entrapped into the nonpolar side chains of proteins. Contrary to this, in our study coarser fraction although showed high protein content but had low OAC, indicating that protein conformation is in such a manner that less hydrophobic groups are exposed on the surface. High OAC of finer fractions with low protein content indicated that proteins in finer flour had more hydrophobic groups exposed for interaction with fat.

The high OAC of the flour fractions is considerably applicable in food stuffs that require taste preservation, enhancement of appetizing properties and prolonged shelf life. These properties are chiefly required in baked, beef and meat products. Therefore, flour fraction of 74 μm particle size having higher OAC can be considered superior to other fractions as flavor retainer and could find application in the food items that require absorption of oil.

3.7. Swelling capacity and swelling index

Swelling capacity of grass pea flour did not change in the particle size range of 249 μm to 105 μm, but further reduction of size to 74 μm caused significant increase in swelling capacity of the flour (Table 5). Proportions of the particles, variety type and various kind of processing techniques or effectiveness are some of the factors on which the swelling capacity of the flour is dependent. Farooq et al., 2018 reported that fine particles of all rice varieties studied by them showed higher swelling ability and coarser particles exhibited the least swelling power because of more hardness and less surface area. The swelling index is an indicative of the propensity of the starch present in the sample to absorb water under specific provisions like temperature and availability of water. Swelling index increased significantly with reduction in particle size of the flour fractions from 249 μm to 179 μm, and the increase became non-significant with further decrease in particle size (Table 5). Swelling index of the particles reflects the degree of binding forces within the particles. The increase in swelling index could be attributed to the improvement in surface area per unit volume, with the reduction in particle size. Higher Swelling index and swelling capacity of 0.43, 227% and 0.44 and 226% was observed for the finer flour fractions of 105 and 74 μm, respectively. Similarly the higher swelling capacity for corn bran of finer particle size has been reported by Singh et al., 2013. Our outcomes are in agreement with the results of Kaur and Singh, 2005 who reported that the average surface area and overall proportion of water retained by fibers vary inversely with the particle size. This may be due to the damage of fibre matrix and collapse of pores during grinding (Auffret et al., 1994). Further it has been stated that the fine particles display elevated packing density.

3.8. Foaming capacity and stability

Increased integration of air bubbles implicit fair capacity to form foam, while the potential of the proteins and other constituents to interact with the air bubbles by the formation of a potent and viscid film around them and limiting the diffusion of air through them, is expressed as foam stability (Ma et al., 2011). Foaming properties are dependent on the proteins, as the surface proteins are highly active and contribute to the formation of foam when agitated. The foaming capacity (FC) of grass pea flours of different particle sizes varied from 56.32 to 81.73% (Table 5). The foaming capacity of 80.7% and 81.73% was observed for flour with particle size of 211 and 180-150 μm, respectively and it decreased significantly with decline in particle size of flour. The foam stability of 83.5% and 82.8% was observed for flour with particle size of 211 and 179 μm, respectively which further decreased significantly with decrease in particle size of flour. Figure 3 represents results of foaming stability as function of time. Stability of foam is considered supreme because whipping agent should be capable to sustain the foam for a longer duration. Kaur and Singh, 2005 elucidated that soluble protein when existent in aqueous phase, exhibit fair foam stability of the flour by virtue of their increased surface characteristics. In our study, flour with particle size 211 and 179 μm showed better FC and FS and might be used in the formulation of protein rich eatables that require aeration in their texture, such as ice creams, cakes and confectionary products.

3.9. Pasting properties

The pasting characteristics of flour depend on behavior of starch molecules during and after heating. The viscosity of gel formed is a major aspect to decide the uses of flour in various applications. The data of pasting properties of grass pea flour with varying particle sizes is presented in Table 6. The results revealed that particle size significantly influenced the pasting properties. Pasting temperature (PT) is the minimum temperature at which flour is cooked. It was maximum for the grass pea flour sample with particle size 249 μm, was almost similar till particle size of 179 μm and then reduced with fall in particle size, which showed that flour with finest particles gelatinize at low temperature. Similar trend in PT have been reported by Ahmed et al., 2016 for chestnut flour. The high values of PT of flour indicate the occurrence of starch which is sustainable to expansion and breakage.

The PV of the flour samples ranged between 0.73-0.98 Pa.s. Peak viscosity increased significantly ($p < 0.05$) as the particle size changed from 180-150 μm to 149 μm and then did not increase significantly with further reduction in particle size of flour. The higher PV (0.98 Pa.s) of 74 μm particle size flour fraction could be attributed to the presence of the fine components *viz.* protein and starch and least proportion of fiber. Bolade et al., 2009 correlated the variations in the PV values of different

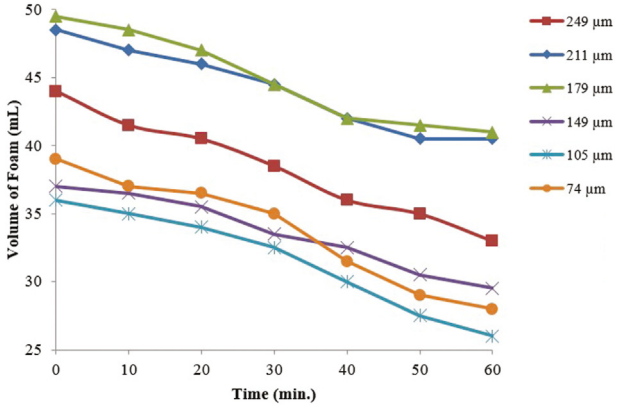

Figure 3. Foam volume of grass pea flour samples of different particle sizes as a function of time.

Table 6. Effect of particle size on pasting parameters of grass pea flour.

Particle Size (μm)	Pasting Temperature (°C)	Peak Time (min.)	Peak Viscosity (Pa.s)	Trough Viscosity (Pa.s)	Final Viscosity (Pa.s)	Breakdown Viscosity (Pa.s)	Setback Viscosity (Pa.s)
249	85.38 ± 0.03^{a}	6.80 ± 0.28^{a}	0.73 ± 0.05^{b}	0.68 ± 0.08^{a}	0.92 ± 0.04^{c}	0.49 ± 0.03^{b}	0.24 ± 0.04^{b}
211	84.53 ± 0.03^{ab}	6.66 ± 0.37^{a}	0.78 ± 0.12^{b}	0.74 ± 0.13^{a}	0.97 ± 0.11^{c}	0.44 ± 0.01^{b}	0.24 ± 0.02^{b}
179	84.53 ± 0.03^{ab}	6.63 ± 0.04^{a}	0.89 ± 0.02^{b}	0.85 ± 0.03^{a}	1.09 ± 0.16^{c}	0.46 ± 0.00^{b}	0.25 ± 0.01^{b}
149	83.70 ± 0.00^{b}	6.50 ± 0.04^{a}	0.97 ± 0.01^{a}	0.93 ± 0.01^{a}	1.17 ± 00.01^{b}	0.42 ± 0.01^{b}	0.25 ± 0.00^{b}
105	84.13 ± 0.53^{b}	6.96 ± 0.04^{a}	0.95 ± 0.00^{b}	0.86 ± 0.03^{a}	1.19 ± 0.01^{b}	0.87 ± 0.00^{a}	0.34 ± 0.00^{a}
74	83.70 ± 0.00^{b}	7.00 ± 0.04^{a}	0.98 ± 0.02^{a}	0.88 ± 0.02^{a}	1.27 ± 0.01^{a}	0.99 ± 0.00^{a}	0.38 ± 0.01^{a}

Results are expressed as mean values ± standard deviations of three replications. Means in a column with same superscripts are not significantly different ($p \leq 0.05$).

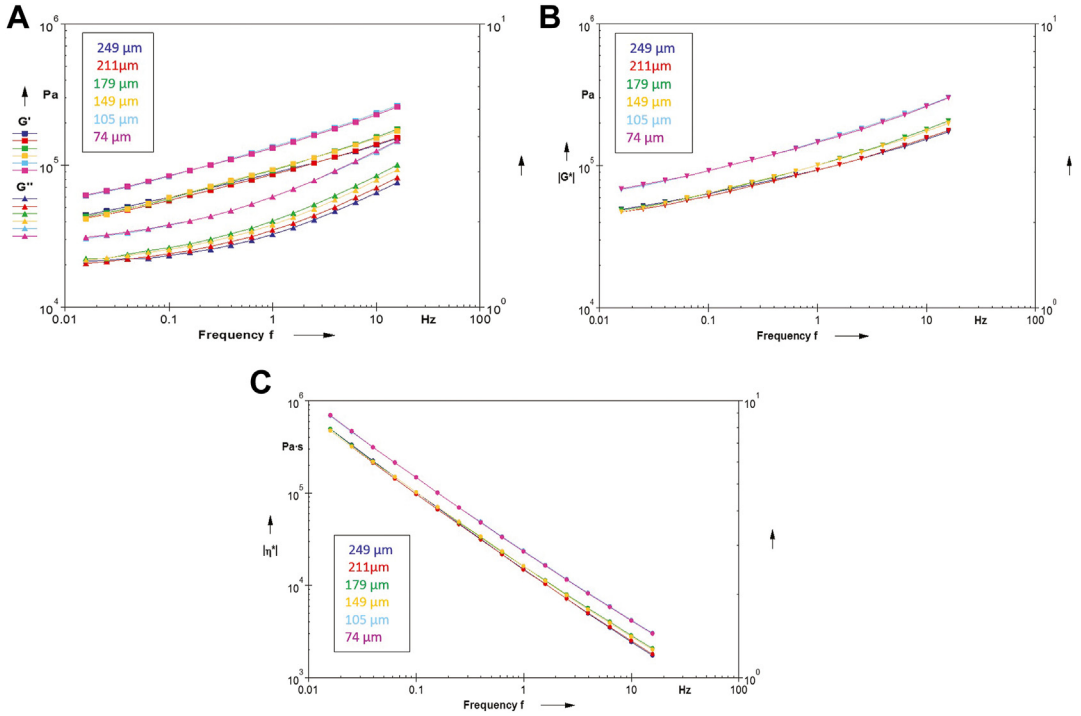

Figure 4. A) Frequency sweep for grass pea flour samples of different sizes as a function of storage (G′) and loss (G″) modulus; B) as a function of complex (|G*|) modulus; and C) as a function of complex viscosity |η*|.

particle size fractions with varied extent of water absorption and swelling capacity of the starch molecules during heating in rapid visco analyzer. Moreover, swelling capacity is associated with the amount of fine constituents present. For the flours having higher values of protein content, there are chances that the swelling may be restricted due to the fact that the water is not within the reach of starch granules which are lodged inside a rigid protein framework.

The trough viscosity (TV) or hot paste viscosity (HPV) of the flour samples did not differ significantly. Final viscosity (FV) or cold paste viscosity (CPV) specifies the paste forming potential of a flour sample. Gradual and significant increase in the final viscosity (FV) was observed with reduction in particle size.

Breakdown viscosity (BV) is an indicative of the extent of dissociation of starch particles caused due to the consecutive deformation at high temperatures and gives indication of paste stability (Cruz et al., 2013). The BV of the flour samples with particle size from 249 μm to 149 μm was almost similar and it increased with decrease in particle size, to 105 & 74 μm. Flour with fine particles having higher values of BV were less resistant to breakdown than coarse particle fractions. Coarser flour having low BD indicates paste formed is quite stable. Setback viscosity (SV) is an indicative of starch retro gradation tendency and gelling ability. SV followed the same trend as was observed for BV. Low SV of coarser flour suggests it is less prone to retrogradation. The flour with lower abilities to retrograde are useful in food stuffs namely sauces and soups (Adebowale and Lawal, 2003). Similar increase in FV, BV, and SV for finer fractions has been accounted by Ahmed et al., 2016 for Indian variety of lentil. The differences in the pasting properties of various fractions could be due to the variability in their components as is evident from Table 1. From the results it can be inferred that high swelling power, PV, SV of fine four fractions may be regarded as the key properties of its starch when flour is to be applied in the preparation of gluten free consumables. While less BD and SV of flour with coarse particle size makes it suitable for use in products requiring good paste stability.

3.10. Rheological measurements of flour dough

In order to optimize product formulation, manufacturing procedures or end product, rheology of dough plays a crucial role. Moreover, rheological properties of dough/suspension are also influenced by the particle size of the flour sample. On the basis of small amplitude measurements, linear viscoelastic region (LVE) for different dough samples prepared from flour samples of grass pea with varying particle size was observed in the range of 0.05–0.1%. The frequency sweeps tests were performed for all the dough samples in the observed LVE region. The significance of the variations in particle size of flour could be seen when frequency

sweep tests were carried out. Figure 4A shows the change in G′ and G″ with respect to the frequency of dough samples prepared from grass pea flour of different particle size. The results revealed that for all the examined dough samples, G′ was greater than G", in the complete sweep of frequencies and both moduli inconsiderably rised with frequency which suggested a solid elastic like response and such types of dough could be categorized as elastic gels (Lai and Liao, 2002). Corresponding inferences on dynamic rheology have been listed for rice, maize flour dough (Gujral and Pathak, 2002; Bala et al., 2018). The dough made up of flour with particle size 249 and 211 μm exhibited the minimum G′ and G″ values at all the evaluated values of frequency and indicated a more viscous and a less elastic behavior. The lower G′ value may be due to deformability of starch granules or lower elasticity of the continuous phase. However, reverse trend was shown by the dough made up of fine particle flour 105 μm and 74 μm with the highest values of G′ and G″ at all frequency. These differences in the rheological behavior of dough could be correlated to the flour composition (protein and starch) as reported by Ahmed et al., 2016.

Complex modulus (|G*|) value provides information about the strength of the dough samples. The effect of differences in particle size of the flour was evident from the results of Figure 4B. |G*| value was highest for dough of the flour with particle size in the range of 105 μm. Our results are in accordance with the findings of Sakhare et al., 2014 who found that wheat dough from the finest fraction (74 μm) indicated the stronger dough features in contrast to other coarser fractions.

Complex viscosity (|η*|) of all the samples decreased as a function of the frequency showing shear thinning and viscoelastic behavior (Figure 4C). Values of Complex viscosity of grass pea flour samples increased with decline in particle size. Our results are according to the general expectation that finer particle size fabricates higher surface area and accords to the greater durability. A reverse trend in complex viscosity and its dependence on particle size of flour has been observed for wheat dough samples (Cristiano et al., 2019) and pumpkin flour suspension (Ahmed et al., 2014). The |η*| value of finer fraction was significantly more than that of coarser flour fraction, which could be attributed to the lower amount of proteins and high amount of carbohydrates. Higher complex viscosity value specifies an increase in the molecular interactions and strengthening of dough structure. On the basis of dynamic rheological studies it is concluded that fine fraction of grass pea flour could be used in the making of dough based products such as breads and Indian flat breads/*Parotta*.

4. Conclusions

Distribution of flour particle size has a crucial impact on its properties and the characteristics of end products. Different properties of grass pea flour were evaluated as a function of decreased particle size from 249 to 74 μm. Our results revealed that protein content decreased and fat content increased, while ash and carbohydrate content did not vary significantly with decrease in particle size of the flour. Particle size distribution data of different flour samples showed mean values were in the span of 20.2–23.7 μm, indicating overall flour was finely ground. SEM revealed oval, round, rectangular and ellipsoidal starch granules, attached to it protein and fiber components were also visible. Flow properties showed reduction in particle size changed flow pattern from good to fair. Water solubility index, swelling capacity, swelling index and oil absorption capacity increased, while the foaming capacity and stability decreased with decrease in particle size of grass pea. Our findings evidenced that pasting and rheological properties were also influenced. Flour with finer particle showed higher PV and FV, G′ G″ and |G*| values. Based on various properties, it can be accomplished that grass pea flour of different particle size can be used to develop novel foods for different end users.

Declarations

Author contribution statement

Manju Bala: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Saksham Handa: Performed the experiments; Wrote the paper.

Mridula D: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

R. K. Singh: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

The authors have not taken permission to share data.

Declaration of interests statement

The authors declare no conflict of interests.

Additional information

No additional information is available for this paper.

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