Physicochemical and microstructural characterisation of green gram and foxtail millet starch gels

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Abstract The starch and starch gels from green gram (GG) and foxtail millet (FM) were characterised for their physicochemical, thermal and microstructural characteristics; the features of shape and size were determined by image analysis. Both GG and FM formed well-set gels at 9% concentration of starch. The fracture strain of the gels was between 78 and 80% indicating non-brittle gels. The peak temperatures of the native flour of GG (74.9 °C) and FM (75.7 °C) were significantly higher than their corresponding starch samples (72.2 and 75.0 °C). The conclusion temperatures of the FM native flour (81.2 °C) and starch (79.4 °C) samples were higher than the native GG flour (79.9 °C) and GG starch (77.1 °C) samples. Starches were nearly spherical as the roundness values were between 0.88 and 0.95. Green gram starch was pentagonal having an average diameter of 3.9–9.2 µm while foxtail millet starch was spherical with a diameter of 4.9–10.1 µm. The freeze-dried GG and FM starch gels showed cellular structure containing organised hexagonal pores, bound by thin pore walls; the GG starch gels deviated from the circular shape as they had the highest elongation value of 4.21. The thicker pore walls were observed for GG starch gels (0.88 µm) compared to that of FM samples (0.57 µm). The higher pore wall thickness in the case of GG gel showed the formation of junction zones.

Keywords Green gram · Foxtail millet · Gel · Microstructure · Image analysis

Introduction

Millets are cultivated in central and eastern Asia, Russia, China, India and many parts of Africa (Amadou et al. 2011) and form a major part of the diet of the people living there. These tiny grains are nutritionally comparable to the popular cereals and have starch as the major component. The health benefit of millet consumption is due to the presence of starch and its characteristics (Singh et al. 2010). Among the common millets, foxtail millet is one of the major millets grown in India though originated from Italy, and hence, it is called the Italian millets. Nagaprabha and Bhattacharya (2016a) have studied the rheological characterisation of sol-to-gel transition of foxtail millet flour gels to identify the gelling temperature. Foxtail millet has the potential for developing health benefiting convenience gelled foods possessing nutraceutical property, and the addition of cations like CaCl_2 and FeSO_4 help to achieve a product with structural integrity (Nagaprabha and Bhattacharya 2016b). However, the literature on the characterisation of millet starch is lesser when compared to that of common cereals. Further, the formation of foxtail millet starch gels is yet to be reported.

The mung bean, also called green gram, is one of the widely used pulses in India; it is also cultivated in China, Southeast Asia, North America, Africa and Australia. It contains the essential nutrients required for the effective functioning of the body (Butt and Batool 2010). It is used in high proportions in infant foods because of good digestibility, high protein content (about 24%), and low levels of anti-nutritional factors and allergens. It is preferred by consumers as the prevalence of diabetes, obesity, and heart diseases are increasing among all age groups of the population.

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Starch is the major component of many economically important food crops such as cereals, legumes, tubers, rhizomes, etc. Structure and physicochemical properties of starch vary widely with its botanical origin. It is isolated and used in many food formulations to improve the functional properties, altering texture and consistency of products. The physical properties such as the gelling of puddings, thickening in gravies, structure to breadcrumb, the setting of cakes, etc. are the common examples of the important attributes of food products. The application of starches thus mainly depends on their physicochemical properties (Adebowale et al. 2005). Characterisation of isolated starch forms the basis for categorising its source and structural features that include diffraction and crystallinity of starch granules (Ratnayake et al. 2001). Sikora et al. (2007) have investigated the attributes of dessert sauces that have been thickened by starch–xanthan gum combinations. Legume starches have been claimed to be the better substrates than cereal and tuber starches due to the absence of pores in the granules, uniformity in granule size and containing less fat (Hoover and Sosulski 1991), and the difference in the amount of ‘B’ type crystallites (Ratnayake et al. 2001). Sashikala et al. (2015) have studied the effect of heat treatment on the protein solubility of the green gram; heat treatment has been reported to increase the insoluble protein fractions. Singh and Ali (2000) have studied the acid degradation of starches from finger millet and green gram with different acids; molecular weight of these starches has been reported to decrease, and HCl/HNO₃ offered the highest reduction.

Gels, being convenient to use and transport, are more preferred to consumers than the liquid based food products. Cereal-based gels are mainly prepared from hydrocolloids like isolated starches while the use of whole grain is rather rare. Preparation of millet-based gels can thus offer a specialty health food. Hence, there is a need to study the process of gel formation using millet flour and the attributes of the gels including sensory acceptance of the product. Gel formation from dispersion is due to the presence of polysaccharides and proteins, the interaction between molecules, forces of attraction such as Van der Waals force, electrostatic force, covalent bonds, etc. A polysaccharide including starch and pectin forms a gel. Some food products are based on cereal/pulse flour of which fabricated gels are a new approach. The common food gels are mostly fruit-based and retain a transparent or translucent appearance. On the other hand, grain-based gels can form a new group of products possessing health benefits; the starting raw material can be the grain flour and offer satiety to the consumers. Thus, the detailed knowledge on the different grain-based and starch-based gels is necessary for developing fabricated biogels. Further, dried gels possess a wide range of new applications and belong

to the category of cellular solids. This type of fabricated structure can be classified according to their open/closed cells, distribution of cells, thickness of cell wall, and size and shape of the cell (Tiwari et al. 2015). Hence, the development and characterisation of flour or starch-based gels using a cereal or legume flour/starch are worthy of investigation as these products can serve as the health-benefitting convenience foods wherein the cost-effectiveness is also expected. Further, the developed gels can also serve as a carrier of nutritional ingredients. An integral component of developing flour/starch-based gels is the isolation and characterisation of the starch that does the gel formation.

Thus, the objectives of the present study were (1) to isolate the starch component from FM and GG, (2) to determine the gel formation characteristics from their starches, and (3) to characterise the starch and gel cellular structure employing image analysis.

Materials and methods

Materials

The procurement of green gram (*Vigna radiata* L.) (GG) and foxtail millet (*Setaria italica*) (FM) was detailed by Nagaprabha and Bhattacharya (2016a); the preparation of flour from FM and GG was also mentioned.

Isolation of starches from green gram (GG) and foxtail millet (FM)

Isolation of starches from green gram and foxtail millet was conducted by following the methods of Madhusudhan and Tharanathan (1995) and Dharmaraj et al. (2014), respectively.

Isolation of starch from green gram (GG)

A laboratory model pulveriser (model # M20, Ika, Germany) was used to grind dehusked split pulse (*dhal*) of GG to obtain the fine powder; the ground sample was passed through a 170 mesh British Standard (BS) sieve having 90 µm aperture. The powder was soaked in water for 12 h and then sieved through a 240 mesh BS sieve (63 µm aperture). The sediment was suspended in dilute NaOH (pH 9.0) for 10 min followed by centrifugation at 3000 rpm for 5 min. The precipitate was washed with water, and then repeatedly washed with 0.1 M NaCl–toluene solution (10:1). The upper layer of the protein–toluene complex was removed by centrifugation at 2000 rpm for 2 min; it was termed as # IV. Supernatant with small granules was removed by the high-speed

centrifugation at 10,000 rpm for 10 min (# III). The sediment starch contained in the soft upper layer (# II) and bottom hard layer (# I) were also collected. All 4 fractions of the (numbered as I, II, III and IV) were collected together, thoroughly washed with deionized water, decanted and again washed with ethanol, acetone, and ether. The samples were finally dried in air, packaged in polyethylene pouches and were used for further studies. The whole process of isolation was repeated twice.

Isolation of starch from foxtail millet (FM)

Polished foxtail millets were steeped in deionized water for 15 h followed by mashing with 5 volumes of water in a laboratory grinder (SITA tilting wet grinder, Coimbatore, India). The paste thus obtained was then passed through a series of sieves like 150 BS (105 μm aperture), 200 BS (75 μm) and 350 BS (45 μm). The residue thus obtained was again dispersed in water, wet ground and sieved. The processes of grinding and sieving were repeated until the residue was free from starch. Crude starch was then dispersed in water and washed with dilute NaOH followed by washing with distilled water and 0.1 M NaCl solution, and centrifuged at 5000 rpm for 10 min. The sediment was collected and treated with toluene (1:5) to denature the protein molecules; the upper layer was removed, washed with water and centrifuged to obtain the starch fraction. The starch fraction was dried at 45 °C for 1 h, packaged in polyethylene pouches and was used for further study. The process of isolation was repeated twice.

The protein, lipid and starch contents of the starch samples were determined following the AOAC (2002) and Goñi et al. (1996) methods. The total starch content was determined by taking 0.1 g of starch sample, and the glucose content was analysed using GOD–POD reagent supplied by Megazyme Total Starch Assay Kit (AA/AMG 11/01) following AACC (2000) method, # 76–13.

Gel from GG and FM starches

The method for gel preparation was detailed by Nagaprabha and Bhattacharya (2016b). Dispersions of 6, 7, 8 and 9% (dry solid basis) were heated in a water bath, maintained at 95 °C, for 15 min for GG starch and at 95 °C for 30 min for FM starch, respectively, with continuous stirring. These conditions were selected after conducting the preliminary trials to obtain well-set gels. Later, the samples were poured into petri plates (35 mm in diameter and 10 mm in height) and allowed to set. After 4 h of setting time, these gels were manually scooped out of the petri plates.

Instrumental texture measurement was performed by employing a texture analyser (model #TAHD, Texture

Analyzer, Stable Microsystems, Surrey, UK) up to a strain of 0.8 employing a crosshead speed of 1 mm s^{-1} . Textural attributes like peak force, energy for compression and sensory texture, and physical properties such as opacity and syneresis were determined for the set gels (Nagaprabha and Bhattacharya 2016b).

The set gels were freeze-dried for 16 h at a temperature of – 24 °C by employing a laboratory model freeze dryer (model # Lyodryer-LT-5S, Lyophilization Systems, New York, USA). The freeze-dried gels were ground to fine powder to obtain Fourier transformation infrared spectra (FTIR) and for determining the thermal properties. The process of gel making, and freeze drying were repeated twice.

The native flour, starch, and starch gel from GG and FM were characterised for their physicochemical, thermal and microstructural characteristics.

Fourier transformation infrared spectra (FTIR)

The FTIR spectra of the native flour, starch and gel flour of the GG and FM samples were obtained on a FTIR spectrophotometer (model # Nicolet 5700, Thermo Electron Corporation, Madison, WI, USA) at the room temperature (about 25 °C). The dry powdered samples were mixed with potassium bromide and then compressed to obtain the thin pellets before analysis. The samples, in triplicates, were scanned in the region between 4000 and 400 cm^{-1} (Liu et al. 2002).

Thermal properties

Thermal properties of the native grain flour, starch, and the gel flour were determined using a differential scanning calorimeter (model # DSC 8000, Perkin Elmer, USA) following the method of Fujita et al. (2003). Sample (4 mg) and distilled water (12 μl) were sealed in an aluminium pan, kept for overnight at 4 °C, and heated from 25 to 100 °C at a heating rate of 10 °C min^{-1} against an empty aluminium pan as the reference. The onset temperature (T_0), peak temperature (T_P) and enthalpy of gelatinisation (ΔH) were obtained by using the software provided by the equipment manufacturer.

Electron microscopy

A scanning electron microscope (model # 435VP, Leo Electron Microscopy, Cambridge, UK) was used to examine the microstructure and shape of the grain flour and starch. The cross-sections of the freeze-dried gel samples were also examined after coating with a thin film of gold employing a sputter coater (Nagaprabha and Bhattacharya 2016a). The samples were examined at an accelerating

voltage of 15 kV at magnifications of 200 $\times$ and 1000 $\times$, and the representative photomicrographs were presented.

Image analysis

The electron photomicrographs of the samples were subjected to image analysis by using the image analysis software (IMAGEJ 1.45 s, National Institute of Health, Maryland, USA). The surface area of pores (S), perimeter (P), roundness, elongation, and thickness of pore wall were obtained and/or calculated from the software, and by employing the Eqs. (1) and (2) (Tiwari et al. 2015; Russ 2011).

$$\text{Elongation} = \frac{F_{\max}}{F_{\min}} \quad (1)$$

$$\text{Roundness} = \frac{4S}{\pi F_{\max}^2} \quad (2)$$

Here, $F_{\max}$ and $F_{\min}$ were the maximum and minimum Ferret dimensions, respectively. Elongation of a body (Eq. 1) is the ratio of the maximum and minimum Ferret dimensions; it reflects the ratio of the length (highest dimension) and breadth (smallest dimension). A circular sample has an elongation value of 1. The value of elongation increases markedly for a highly elongated body like a thread. The roundness (Eq. 2) indicates the circularity of a sample. A typical circle possesses a value of 1, and any deviation from the circular shape is recognized by its deviation from 1.

Data analysis

The statistical significance of the multiple comparisons of samples was made by employing Duncan's multiple range test (DMRT) at $p \leq 0.05$ using the statistical software Statistica'99 (StatSoft, Tulsa, OK, USA).

Results and discussion

Isolation of starch and gel formation

The starch and protein contents of the GG and FM starches were 94 and 95, and 0.5 and 0.7%, respectively; the moisture and lipid contents were 5.3 and 0.2, and 4.0 and 0.3%, respectively.

The desirable characteristics of a food gel are the attractive appearance, quick and well setting ability, and maintenance of the shape during the post-gelling phase. The 8 and 9% concentrations of GG and FM starches were necessary to form acceptable gels with adequate integrity; a lesser concentration of solids offers a fluid-like weak gel.

The texture is a critical physical property that affects the sensory acceptability of the developed gels. In the present study, compression characteristics which simulate the eating inside the mouth were performed. The force–deformation curves showed fracture only when a high extent of compression was employed; the phenomenon of fracture was exhibited by a drop in the force while compression was in progress. The fracture strain of the flour gels was between 78 and 80% to indicate the formation of non-brittle gels (Fig. 1) which exhibited delayed fracture. It may be mentioned here that a low value of fracture strain indicates the phenomenon of early fracture/failure at a low deformation of the sample; it is a desirable feature for a crisp product like the biscuit. The GG and FM starch gels at 8 and 9% concentrations of starch did not show any significant difference ($p \leq 0.05$) in the values of peak force and compression energy (Table 1). The peak force indicated the maximum force required by the mouth to achieve the desirable deformation under compression. Besides, the compression energy was an indicator of the extent of energy to be spent while compressing.

Fig. 1 Sample compression curves for green gram flour gels at 10% concentration (top), and foxtail millet flour gels at 11% concentration (bottom)

Syneresis of water from the prepared gel is a natural but undesirable phenomenon during which the unwanted excess water comes out of the already formed gel matrix; finally, it affects the appearance of the gel and reduces the shelf-life. The syneresis of the developed gels was low (between 1.0 and 2.4%). The FM gels showed more opacity (98–99%) compared to GG gels (94–95%), and this might be due to the difference in the starch characteristics of the millet and pulse samples (discussed latter). Food gels are usually belonging to one of the three categories such as transparent, translucent and opaque. However, gels obtained in the present study were all opaque in appearance.

Thermal properties

The thermal parameters offered by a DSC curve are the initial peak temperature (obtained from the DSC curve when the measured values start deviating from the base line), peak onset temperature (where the line drawn through the near linear portion of the ascending peak slope intersects the baseline) and peak completion temperature (when the line through the descending peak slope intersects baseline) (Brown 2004). Table 2 shows the thermal characteristics of GG and FM native flours, starches and gel flours. The onset temperatures (T_0) of the FM flour and starch samples (70.7 and 70.4 °C, respectively) were marginally higher (significant at $p \leq 0.05$) than the corresponding GG (70.0 and 67.9 °C) samples. The peak temperatures (T_p) of the native flour of GG (74.9 °C) and FM (75.7 °C) were significantly greater ($p \leq 0.05$) than their corresponding starch samples (72.2 and 75.0 °C for GG and FM, respectively). The conclusion temperatures (T_c) of the FM native flour (81.2 °C) and starch (79.4 °C) samples

were higher ($p \leq 0.05$) than that of native GG flour (79.9 °C) and GG starch (77.1 °C) samples. The differences in the T_0 , T_p and T_c values between the GG and FM samples might be due to the inherent differences in size, form, distribution of starch granules and internal arrangement of starch fractions within the granule (Sandhu and Lim 2008). The gelatinisation temperature range ($T_c - T_0$) of the native flours of the GG (9.9 °C) and FM (10.6 °C) was significantly higher ($p \leq 0.05$) than their corresponding starches. This was probably because of other components such as protein and fat which might have affected the phenomenon of gelatinisation of the crystalline region of the flour, and hence, the removal of the other components significantly decreased the temperature ranges. The endothermic enthalpies (ΔH) of the GG and FM starches (7.1 and 6.4 J/g) were higher ($p \leq 0.05$) than their corresponding native flour samples (4.2 and 3.1 J/g, respectively). The higher ΔH values for the starch samples might be due to the higher extent of gelatinisation in the starch samples than their native flours. A high gelatinisation temperature indicated that an increased level of energy was required to initiate the process of starch gelatinisation. Higher transition temperatures, resulting from more crystallinity, changed the gelatinisation enthalpy of starches due to the loss of molecular arrangements within the granules (Tester and Morrison 1990). Kim et al. (2009) and Fujita and Fujiyama (1993) investigated the thermal properties of foxtail millet and reported similar results for the native millet sample. Gelatinisation temperatures of both GG flour and GG starch exhibited similar range with that of lentil starch (Kaur et al. 2010) and different legume starches (Sandhu and Lim 2008).

No gelatinisation peaks were observed for the gel flour. Instead, retrogradation peaks were noticed for the GG and

Table 1 Effect of concentration of green gram (GG) and foxtail millet (FM) starch on gel characteristics

Concentration of starch (%)		Peak force (N)	Energy for compression (mJ)	Fracture strain (%)	Opacity (%)	Syneresis (%)	Sensory texture
GG	6	20.1 ± 0.7 ^c	33.2 ± 1.6 ^c	No fracture	94.4 ± 0.5 ^a	2.4 ± 0.1 ^d	Soft gel
	7	21.5 ± 0.9 ^c	34.3 ± 0.4 ^c	70.2 ± 1.5 ^a	95.1 ± 0.6 ^a	1.9 ± 0.1 ^c	Weak gel
	8	24.9 ± 1.4 ^d	37.0 ± 1.5 ^d	78.9 ± 2.0 ^b	95.3 ± 0.4 ^a	1.5 ± 0.0 ^b	Acceptable gel
	9	26.9 ± 1.1 ^d	40.2 ± 1.5 ^d	80.2 ± 1.5 ^b	95.2 ± 0.8 ^a	1.0 ± 0.1 ^a	Slight hard gel
FM	6	4.6 ± 0.0 ^a	6.4 ± 0.0 ^a	No fracture	–	–	Very soft gel
	7	18.2 ± 0.8 ^b	21.5 ± 1.3 ^b	No fracture	–	–	Weak gel
	8	23.6 ± 0.1 ^d	37.3 ± 1.7 ^d	78.2 ± 1.1 ^b	99.5 ± 1.1 ^b	1.5 ± 0.2 ^b	Acceptable gel
	9	25.5 ± 1.8 ^d	39.7 ± 0.7 ^d	79.0 ± 2.4 ^b	97.9 ± 0.8 ^b	1.1 ± 0.1 ^a	Slightly hard gel

Not determined

Values in the same column with different superscripts are significantly different at $p \leq 0.05$

Table 2 Thermal properties of native flour, starch isolates and gel flour of foxtail millet and green gram

Sample	Onset temperature, T_0 (°C)	Peak temperature, T_p (°C)	Conclusion temperature, T_c (°C)	$T_c - T_0$ (°C)	Enthalpy, ΔH (J/g)
GG native flour	69.96 ± 0.31^d	74.89 ± 0.21^d	79.89 ± 0.11^e	9.93 ± 0.07^e	4.16 ± 0.03^d
GG starch	67.90 ± 0.11^c	72.19 ± 0.13^c	77.12 ± 0.01^c	9.22 ± 0.03^d	7.05 ± 0.02^f
GG gel flour*	55.13 ± 0.09^b	58.47 ± 0.02^b	64.20 ± 0.02^b	9.07 ± 0.10^c	0.64 ± 0.01^b
FM native flour	70.67 ± 0.06^f	75.71 ± 0.02^e	81.24 ± 0.05^f	10.56 ± 0.01^f	3.05 ± 0.03^c
FM starch	70.40 ± 0.02^e	75.02 ± 0.11^d	79.42 ± 0.02^d	8.57 ± 0.04^b	6.43 ± 0.11^e
FM gel flour*	55.42 ± 0.10^a	58.38 ± 0.04^a	60.94 ± 0.03^a	5.52 ± 0.09^a	0.15 ± 0.0^a

*No gelatinisation peaks were observed due to complete gelatinisation of the gel flour instead retrogradation peaks were observed

Values of the same column with different superscripts are significantly different at $p \leq 0.05$

FM starch samples, possibly due to the completion of gelatinisation process of the starch during the process of gel preparation. The values of T_0 , T_p and T_c for the retrograded gel samples were lower than that obtained from the gelatinisation endotherm. The melting of the retrograded starch (T_0) in the gel sample was 55.1 and 55.4 °C for GG and FM gels, respectively. The conclusion temperature of the GG gel flour was higher (significant at $p \leq 0.05$) than that of the FM sample. Similar results had been observed for finger millet during hydrothermal treatment (Butt and Batool 2010). The range of melting temperature ($T_c - T_0$) gave an indication of the quality and heterogeneity of the re-crystallized amylopectin. Retrogradation enthalpy (ΔH) reflected the unravelling and melting of the double helices formed during storage, which was a function of amylopectin unit chain length distribution (Butt and Batool 2010).

The difference in the thermal characteristics such as the initial peak temperature, peak onset temperature, peak completion temperature, gelatinisation temperature and enthalpy of the flour and starches of foxtail millet and green gram were due to variations in their composition, and molecular weight and molecular structure of the components. In turn, the thermal properties affect the textural attributes and other physical properties of the developed gels.

FTIR

FTIR spectra revealed the structural organisation of starch chains near the surface of the starch granules. Figure 2A (a–c) shows the FTIR spectra of the green gram native flour, starch, and starch gel flour, respectively. Characterisation of C–H and O–H stretch can be readily accomplished by spectral analysis. The maximum O–H stretching mode was observed in the case of starch from GG

compared to the native flour and gel flour in the region of 3000 and 3600 cm^{-1} of the FTIR spectra. Changes were observed in the O–H stretching regions of water molecule (3000–3600 cm^{-1}) possibly due to the differences in the water content of the GG flour, starch and gel samples. FTIR spectrum of starch showed C–H stretching modes in the 2800–3000 cm^{-1} region which could be attributed to the variations in the contents of amylose and amylopectin in these starches.

GG native flour showed two peaks at 988 and 1044 cm^{-1} that were assigned to the vibrations of C–H bond and C–N stretch, respectively which were absent in the starch and gel flour of GG. Spectra of samples exhibited complex vibrational modes at the low wave numbers (below 800 cm^{-1}) due to the skeletal mode vibrations of the glucose pyranose ring. Water absorbed in the amorphous region of starches was identified as a broad infrared band with a peak at 1637 cm^{-1} (Santha et al. 1990). As the crystallinity of starch increased, this band became weaker in the infrared spectra; for most of the crystalline celluloses, the band at 1637 cm^{-1} was rarely observed (Santha et al. 1990). The finding supported the hypothesis that the 1637 cm^{-1} band was a result of the vibrations of water molecules absorbed in the non-crystalline region of starch. The band observed at 1636 cm^{-1} could be attributed to the water absorbed in the amorphous region of the starch granules.

The FTIR spectra of foxtail millet native flour, starch, and gel flour samples were presented in Fig. 2B (a–c). Of all the flours, there was a strong absorption band in the range of 3311, 3350 and 3380 cm^{-1} which could be attributed to the O–H stretching of the starch. Increased width of the FM starch and the gel flour indicated the formation of inter- and intra-molecular hydrogen bonds. The absorption peak of the native flour changed from 3311 to 3350 cm^{-1} for starch, and 3380 cm^{-1} for the gel flour.

Fig. 2 FTIR pattern of *a* native flour, *b* starch, and *c* starch gel flour of **A** green gram, and **B** foxtail millet

The wider peak of the FM starch indicated the high extent of interactions among the starch molecules and with other molecules that led to the formation of a strong gel network (Sun et al. 2014). Strong peaks at 2926.5 and 2152.0 cm⁻¹ were observed for all the flours showing the asymmetric stretching of C–H and C≡C indicating the presence of

hydrocarbon chromophores (Pal et al. 2008). Starch and gel flour showed a minor change at the same peak compared to the native flour. Some stretching vibrations at 2856, 1742 and 1550 cm⁻¹ that were absent in starch were observed for the native as well as gel flour strengthening the C–H, C=O and N–H bonds. It might be due to the presence of

non-starch constituents such as protein and fat in the native and gel flours. A shifting of C–H bond from 1460 cm^{-1} in native flour to 1420 cm^{-1} in starch, and to 1408 cm^{-1} in gel flour was observed possibly because of the presence of the non-starch constituents in the native flour and gelatinised gel flour.

Electron microscopy and image analysis

Figure 3a, c, e and b, d, f show the photomicrographs of the native flours, starches, and the freeze-dried gels prepared using GG and FM starches, respectively. The native flour of GG and FM exhibited starch granules possibly with adhering other components like the cell wall, fat and protein bodies (Fig. 3a, b). GG starch was pentagonal having an average diameter of $3.9\text{--}9.2\text{ }\mu\text{m}$ (Fig. 3c) while foxtail millet starch was spherical with a diameter of $4.9\text{--}10.1\text{ }\mu\text{m}$

(Fig. 3d). Kim et al. (2009) had also measured the size of foxtail millet starch; the reported values were $10.1\text{--}25.0\text{ }\mu\text{m}$ in length and $4.7\text{--}12.5\text{ }\mu\text{m}$ in diameter. GG and FM starch gels (Fig. 3e, f) showed cellular structure containing organised hexagonal pores, bound by thin pore walls.

The results of image analysis are shown in Table 3; the values of the area, perimeter, roundness, elongation, and thickness of the pores were reported. Starches were nearly spherical as the roundness values were between 0.88 and 0.95 against the ideal circular shape of 1.00. The cellular structure of dried GG starch gels deviated from circular shape as they possessed the highest elongation value of 4.21.

Thicker pore walls were observed for GG dried starch gels ($0.88\text{ }\mu\text{m}$) compared to that of FM samples ($0.57\text{ }\mu\text{m}$). The area and perimeter of GG gels were also higher

Fig. 3 Photomicrographs of **a** GG native flour, **b** FM native flour, **c** GG starch, **d** FM starch, **e** GG gel, and **f** FM gel

Table 3 Microstructural characteristics of the starches and starch-gels of green gram (GG) and foxtail millet (FM)

Sample	Area (μm^2)		Perimeter (μm)		Major dimension (μm)		Minor dimension (μm)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
GG Starch flour	18.4–51.8	42.6 $\pm$ 14.1 ^a	16.2–28.1	24.8 $\pm$ 4.6 ^a	5.3–8.7	7.7 $\pm$ 1.3 ^a	4.2–7.5	6.6 $\pm$ 0.9 ^a
GG Starch gel	169.9–400.9	283.4 $\pm$ 166.2 ^b	55.9–83.3	69.6 $\pm$ 19.4 ^b	19.8–28.2	23.9 $\pm$ 5.8 ^b	10.8–17.7	14.3 $\pm$ 4.9 ^b
FM Starch flour	15.8–38.9	30.2 $\pm$ 12.6 ^a	15.2–23.4	20.4 $\pm$ 4.5 ^a	4.6–7.2	6.2 $\pm$ 1.4 ^a	4.8–6.8	5.9 $\pm$ 1.3 ^a
FM Starch gel	27.5–73.1	43.6 $\pm$ 25.6 ^a	25.5–34.0	28.6 $\pm$ 4.7 ^a	7.9–10.9	9.2 $\pm$ 1.5 ^a	4.9–6.3	5.6 $\pm$ 0.7 ^a
Sample	Roundness (–)		Compactness (–)		Elongation (–)		Cell wall thickness (μm)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
GG Starch flour	0.82–0.91	0.88 $\pm$ 0.07 ^b	0.32–0.82	0.35 $\pm$ 0.11 ^a	1.33–3.33	3.06 $\pm$ 0.23 ^a	–	–
GG Starch gel	0.54–0.64	0.59 $\pm$ 0.06 ^a	0.23–0.38	0.31 $\pm$ 0.08 ^a	2.91–5.50	4.21 $\pm$ 1.82 ^a	0.83–0.95	0.88 $\pm$ 0.16 ^b
FM Starch flour	0.94–0.96	0.95 $\pm$ 0.05 ^b	0.32–0.71	0.48 $\pm$ 0.20 ^a	1.48–3.22	2.41 $\pm$ 0.91 ^a	–	–
FM Starch gel	0.61–0.78	0.67 $\pm$ 0.10 ^a	0.31–0.39	0.36 $\pm$ 0.11 ^a	2.68–3.33	2.95 $\pm$ 0.31 ^a	0.26–0.94	0.57 $\pm$ 0.35 ^a

–, Difficult to measure

Values of the same column with different superscript letters differs significantly at $p \leq 0.05$

compared to that of FM samples. Biggest pores were observed with the cellular structure of freeze-dried GG starch gels; The highest perimeter was thus associated with this sample. The higher pore wall thickness for GG gel showed the formation of junction zones (Table 3). These led to a stronger gel network for GG compared to that of FM (Funami et al. 1999). Formation of a thin pore wall and junction zone usually led to a softer gel. Possibly, the difference in the structure and composition of the two grains, i.e., the ingredients present led to a variation in the thickness of the pore wall of the gel (Tiwari et al. 2015).

Conclusion

The starches of GG and FM formed well-set gels. The freeze-dried GG and FM starch gels showed the uniform three-dimensional cellular structure with organised hexagonal pores. GG starch was pentagonal while FM starch exhibited a spherical geometry. FM gels showed more opacity compared to GG gels. Gels from green gram showed distinct gel structure properties of having thicker pore walls and provided stable gel structure with better integrity. These features of GG starch and gel may facilitate their utilisation as food ingredients in various food products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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