



Impact of moisture content on microstructural, thermal, and techno-functional characteristics of extruded whole-grain-based breakfast cereal enriched with Indian horse chestnut flour

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ABSTRACT

The use of non-conventional seed flour is of interest in obtaining healthy breakfast cereals. The research aimed to study the physico-functional, bioactive, microstructure, and thermal characteristics of breakfast cereals using scanning electron microscopy, X-ray diffractometry, and differential scanning calorimeter. The increase in feed moisture content (16 %) enhanced the bulk density (5.24 g/mL), water absorption index (7.76 g/g), total phenolic content (9.03 mg GAE/g), and antioxidant activity (30.36 %) having desirable expansion rate (2.84 mm), water solubility index (48 %), and color attributes. The microstructure showed dense inner structures with closed air cells in extruded flours. Extrusion treatment rearranged the crystalline structure from A-type to V-type by disrupting the granular structure of starch, reducing its crystallinity, and promoting the formation of an amylose–lipid complex network. Increasing conditioning moisture enhanced the degree of gelatinization (%), peak gelatinization temperature (T_p), and starch crystallinity (%) and reduced the gelatinization enthalpy (ΔH_G) and gelatinization temperature ranges. The results reported in this study will help industries to develop innovative and novel food products containing functional ingredients.

1. Introduction

Generally, malnutrition is the prime cause of immunodeficiency across the globe, affecting infants, children, adults, and older people (Shaly et al., 2022). In children, under-nutrition is characterized by deficiencies in micronutrients and growth failure, and over-nutrition, overweight, and obesity are common issues nowadays because of the consumption of unhealthy diets like junk foods, a diet high in *trans*-fats, saturated fats, sugars, and low in fiber, etc. (Brglez et al., 2022). Ready-to-eat products contain high starch content or refined cereal-based flour, and most are comparatively high in fat, sugar, and salt; thus, they are known for being energy-dense products with a poor nutritional profile. In India, the ready-to-eat industry has gained a significant change with

the increase in demand for the development of sustainable, eco-friendly, and healthy convenience food products (Grasso, 2020).

Extrusion cooking, under high temperature, pressure, and short time, is used to develop a wide range of extruded products such as breakfast cereals, snacks, porridge, puffed snacks, and fiber-rich products. It has several benefits over traditional processes, such as retaining nutritional quality, improving functional attributes, high expansion, and low density with uniform microstructure and crunchy texture (Alam et al., 2016). The quality of extrudates depends mainly on the process conditions of the extrusion system. The positive or negative influence of feed moisture content on the expansion, density, porosity, rehydration ratio, etc., depends on the moisture content levels (Kesre, & Masatcioglu, 2022). Whole grain flour is usually used to develop extrudates with low

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density, high expansion, and crispy structure (Rolandelli et al., 2020). Furthermore, this process allows the addition of other flours, like whole wheat (Oliveira, Schmiele, & Steel, 2017), barley (Jabeen et al., 2022), oat (Brahma, Weier, & Rose, 2016), rice (Gulzar et al., 2021), amaranth (Espinosa-Ramirez et al., 2021), and corn (Zhao et al., 2021).

Whole corn flour is an excellent source to produce ready-to-eat products with high textural characteristics but exhibits low fiber and protein content. Some other whole grains, such as entire barley flour and whole wheat flour, are industrially essential sources of healthy food ingredients because of their high protein, fiber, starch, vitamins, minerals, and antioxidant content (Allai et al., 2021). Besides whole grain flour, other ingredients such as non-conventional seed flour can be blended for the development of an ample range of extruded products (Beigh et al., 2019; Jabeen et al., 2021; Saklani, Kaushik, & Kumar, 2021), with the concern of improving nutritional, and functional attributes of the developed product, which make them suitable to complement whole grains.

Indian horse chestnut (*Aesculus indica*) is known as one of the non-conventional and under-utilized nuts and a good source of fiber, resistant starch, minerals, essential oils (oleic), vitamins, low glycemic power, and bioactive compounds such as quercetin, carotenoids, kaempferol, anthocyanins, phenolics, and flavonols (Allai et al., 2022), which are concentrated in the seed. The seeds contain anti-nutritional factors such as saponins and tannins, which are poisonous and bitter if consumed without processing (raw). So, the seeds must be pretreated and then ground to a fine flour to partially replace the wheat flour (Rafiq et al., 2021). IHCF has excellent therapeutic properties, including anti-pyretic, anti-viral, anti-obesity, anti-inflammatory, etc. (Ahmad & Gani, 2021). These characteristics make IHCF cost-effective, novel, and exciting ingredients for developing extruded breakfast cereals. Due to its excellent therapeutic and nutritional quality, IHCF may be considered a potential non-conventional alternative to conventional flours.

Therefore, this research aimed to evaluate the effect of different levels of extrusion moisture (12–16 %) on the physical, functional, antioxidant, thermal, and microstructural characteristics of whole-grain-based breakfast cereal enriched with IHCF.

2. Materials and methods

2.1. Materials

Barley (PL 807), white corn (DT-2), and wheat (SW-2) were procured from Kargil, India, and SKUAST-K, Shalimar, J&K, respectively. Milling was done to obtain whole barley flour (WBF), whole wheat flour (WWF), and whole corn flour (WCF). The flours were then packed and stored at -21°C until further use. Indian horse chestnut seeds (*Aesculus indica*) were collected manually from the local area of Shalimar, Jammu & Kashmir, India.

Table 1

Physical, techno-functional and bioactive properties of extrudates produced with varying levels of conditioning moisture.

Samples	Conditioning Moisture (%)	Physical characteristics		Techno-functional properties		Bioactive properties	
		SE (mm)	BD (g/mL)	WAI (g/g)	WSI (%)	Total phenolic content (mg GAE/g)	DPPH (%)
E-12	12	2.84 ± 0.22 ^a	4.95 ± 0.11 ^c	6.73 ± 0.12 ^c	48 ± 0.16 ^a	5.87 ± 0.16 ^c	18.33 ± 0.35 ^c
E-13	13	2.81 ± 0.17 ^a	5.05 ± 0.33 ^d	7.04 ± 0.24 ^d	47.5 ± 0.11 ^b	6.41 ± 0.22 ^d	22.21 ± 0.38 ^d
E-14	14	2.77 ± 0.21 ^b	5.11 ± 0.45 ^c	7.35 ± 0.34 ^c	47.1 ± 0.25 ^c	7.33 ± 0.27 ^c	25.44 ± 0.32 ^c
E-15	15	2.74 ± 0.09 ^b	5.17 ± 0.32 ^b	7.58 ± 0.08 ^b	46.2 ± 0.22 ^d	8.25 ± 0.17 ^b	28.73 ± 0.24 ^b
E-16	16	2.7 ± 0.24 ^{bc}	5.24 ± 0.04 ^a	7.76 ± 0.02 ^a	46 ± 0.13 ^d	9.03 ± 0.19 ^a	30.36 ± 0.18 ^a

SE = Sectional expansion rate; BD = bulk density; WAI = water absorption rate; WSI = water solubility index; E-12 (extrudates with conditioning moisture of 12 %), E-13 (extrudates with conditioning moisture of 13 %), E-14 (extrudates with conditioning moisture of 14 %), E-15 (extrudates with conditioning moisture of 15 %), and E-16 (extrudates with conditioning moisture of 16 %). Values are shown as mean ± S.D; Values with different superscripts within same column differ significantly ($p < 0.05$).

2.2. Preparation of Indian horse chestnut flour (IHCF)

The IHCF was prepared according to our previous work (Allai et al., 2022).

2.3. Extrusion process

The breakfast cereals were developed with a constant blend of WWF, WBF, WCF, and IHCF in the ratio of 10:10:77.5:2.5 %. All the ingredients were mixed in a planetary mixer for 15 min. The flour was preconditioned under different moisture content levels (12 % to 16 %) (Table 1). After preconditioning, the moist samples were allowed to equilibrate in a plastic bag and stored for 24 h at 4°C before extrusion.

Extrudates were prepared in a co-rotating twin-screw extruder (Basic Technology Pvt. Ltd., Kolkata, India) with a length-to-diameter ratio of 8:1 and die diameter of 3.0 mm. Throughout the experimentation, the barrel temperature and screw speed were maintained at a constant rate of 130°C and 380 rpm, respectively. After extrusion, the samples were cooled and packed in HDPE bags and stored at ambient temperature (22°C) until further analysis.

The extrudates were specified as E-12 (extrudates with conditioning moisture of 12 %), E-13 (extrudates with conditioning moisture of 13 %), E-14 (extrudates with conditioning moisture of 14 %), E-15 (extrudates with conditioning moisture of 15 %), and E-16 (extrudates with conditioning moisture of 16 %).

2.4. Physical properties of extrudates

2.4.1. Bulk density (BD, g mL^{-1})

The BD was measured as a ratio of the mass of extrudates to its volume (Eq. 1) (Oliveira, Schmiele, & Steel, 2017) using the following formula. Fifteen measurements were performed for each sample, and the average was recorded.

$$\text{Bulk density} \left(\frac{\text{g}}{\text{mL}} \right) = \frac{\text{weight of the sample (g)}}{\text{volume of the sample after tapping (ml)}} \quad (1)$$

2.4.2. Sectional expansion (SE)

The SE of extrudates was defined as the association between the extrudate diameter and the die diameter (mm). Ten measurements were taken using a digital vernier caliper (Mitutoyo absolute, Model No: CD-6 CSX, Made in Japan), and the average was taken (Brennan et al., 2008).

$$SE (\text{mm}) = \frac{\text{Averagediameterofextrudate}}{\text{Die diameter}} \quad (2)$$

2.5. Techno-functional attributes of extrudates

2.5.1. Water absorption indices (WAI) and water solubility index (WSI)

WAI and WSI of extrudates were measured according to Ek, Gu, & Ganjyal, (2021) method with slight modifications. Briefly, 1.5 g of finely powdered sample was placed in a pre-weighed centrifuge tube and filled

with 20 mL distilled water. After intermittent mixing for 5 min at room temperature (22 °C), the tubes containing samples were centrifuged for 10 min at 3000g. The supernatants were carefully transferred to the pre-weighed aluminum cans and dried in an oven at 70 °C till constant weight was achieved. All the readings were taken in triplicates. The remaining gel was weighed and WAI and WSI were calculated as given in Eq. III. and Eq. IV.

$$\text{WAI (g/g)} = \frac{\text{Weight of sediment (wet basis) (g)}}{\text{weight of sample (g)}} \quad (3)$$

$$\text{WSI (\%)} = \frac{\text{weight of solids dissolved in the supernatants (dry basis)}}{\text{weight of sample}} \times 100 \quad (IV)$$

2.6. Color analysis

The extrudates were grounded in a fine powder using a Foss mill for 60 sec. The color evaluation of ground samples was analyzed by measuring the CIELAB space parameters represented as L^* (lightness/darkness), a^* (redness/greenness), and b^* (blueness/yellowness) values by using a Hunter Lab colorimeter (CR 300, Konica Minolta, Japan). The procedure was repeated five times for each sample, and the average was reported.

2.7. Total phenolic content

The procedure of Cheng et al., (2020) was used to calculate the total phenolic content in extrudates. Methanol as a solvent was used for the extraction process. The method uses a Folin-Ciocalteu reagent, 7.5 % Na_2CO_3 , and a spectrophotometer. A calibration curve was made with gallic acid, and the total phenolic content was expressed as mg GAE/100 g of dry sample.

2.8. Antioxidant activity

The DPPH radical scavenging assay estimated the antioxidant activity of the samples according to the method of Zhang et al., (2018). DPPH radical scavenging activity was calculated using equation Eq. V.

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}} \times 100}{A_{\text{sample}}} \quad (5)$$

where A_{control} and A_{sample} are the absorbances of control and sample, respectively.

2.9. Thermal properties

The thermal properties of whole grain-based extrudates enriched with IHCF were assessed as per the method of Gulzar et al., (2021) using a differential scanning calorimeter (Mettler Toledo). 3 mg samples were placed in DSC pans. The samples were then wetted with distilled water in the ratio of 1:2 (flour: water). The samples were run from 20 °C to 150 °C at a heating rate of 10/min, and thermograms were analyzed using Origin Pro 12 software.

$$\text{Degree of gelatinization (\%)} = \left[1 - \left(\frac{\Delta H(\text{extrudate})}{\Delta H(\text{native})} \right) \right] \times 100$$

2.10. Scanning electron microscopy (SEM)

The microstructures of native flours, their blend, and extrudates were studied by using a scanning electron microscope (JSM-6510LV, JEOL, Japan) as per the method given by Pandey et al., (2021). The sample was dehydrated, grounded, placed on an aluminum stub, adhered to double-sided stick tape, and coated with a thin layer of gold. Micrographs were taken at an accelerated voltage of 15 kV and 500 and

1000 magnification levels for morphological characterization.

2.11. X-ray diffraction (XRD)

The samples were grounded with a Foss mill to obtain a fine powder and analyzed using an X-ray diffractometer (D8 Advance, Bruker, Germany). The sample was first sieved through a 45 μm mesh size to get a good signal-to-noise ratio. Diffractograms were taken at an ampere and voltage of 30 mA and 40 kV, respectively, at an ambient temperature. A scanning range between 0° and 40° was used to analyze the data at a scan rate of 1.2°/min. The percentage of crystallinity data of the XRD was processed using the Origin Pro 12 software package by using the following equation:

$$\text{Crystallinity (\%)} = \frac{\text{Area under the peak}}{\text{Total area under the diffractogram}} \times 100 \quad (6)$$

2.12. Statistical analysis

The experiment results were analyzed in triplicates, and the data were recorded as mean \pm standard deviation. Tukey's t -test ($p < 0.05$) was used to compare the results through analysis of variance (ANOVA).

3. Results and discussion

3.1. Physical properties

3.1.1. Sectional expansion (SE)

SE represents the degree of puffing of snacks. The puffing of snacks indicates more lightness and crispiness of extrudates. Additionally, gelatinization of starch is a key parameter in determining the expansion of extrudates. The higher the gelatinization rate, the greater the degree of expansion. The data showed that the SE of extrudates ranged from 2.7 to 2.84 mm (Table 1). E-12 had the highest SE value among tested samples, followed by E-13, E-14, E-15, and E-16. Relatively higher moisture levels soften the molecular structure of amylopectin and reduce the dough elasticity during extrusion, reducing the expansion rate (Ding, Ainsworth, Tucker, & Marson, 2005). Previous literature reported that flours with high fiber and feed moisture content reduced the expansion ratio in cereal-based extrudates (Liu, Hsieh, Heymann, & Huff, 2000; Van der Sman and Broeze, 2013; Oliveira, Schmiele, & Steel, 2017; Seal et al., 2021; Kaur et al., 2022). In concurrence with these authors, reduction in feed moisture content induces drag forces that enhance die pressure, resulting in more expansion of extrudates. The SE directly relates to the screw speed, as increased pressure in the chamber causes superheating that results in quick evaporation of moisture at the exit of the die due to sudden pressure drop and thus increases expansion. Moreover, fiber content both soluble (corn starch and β -glucan in barley) and insoluble fractions (wheat bran and cellulose, hemicelluloses, and lignin in corn) exhibited higher water-binding properties that tend to absorb more water, thus decreasing its accessibility for expansion. Also, the increased viscosity of mass restricts the expansion, which allows the starch to undergo glass transition during extrusion, leading to disintegration of the mixture and decreasing the expansion by inhibiting bubble formation and consequently reducing cell extensibility by puncturing the cell wall.

3.1.2. Bulk density (BD)

The bulk density of flour is an essential parameter in the post-production of extrudates. It provides information for handling, processing, storing, and packaging extruded products (Chisenga et al., 2019). Significant differences were observed in the BD values of samples ranging from 4.95 to 5.24 g/mL (Table 1). Nevertheless, increased BD values could be observed for E-16, followed by E-15, E-14, E-13, and E-12, which could be due to their higher moisture. Feed moisture content is the major parameter affecting the density of extrudates (Oliveira,

Schmiele, & Steel, 2017). Higher moisture content exhibited an elevated density of extruded products as water acts as a lubricant of the amorphous regions of starch molecules, thereby reducing the rheological characteristics of the melt and contributing directly to the gelatinization of starch. High water content also decreases the frictional force between the screw and the blended mixture, which increases the bulk density with reduced expansion (Bisharat et al., 2013). From the previous research, using ingredients containing high fiber content with starch-based extrudates enhanced the density irrespective of the source and fiber content (Arivalagan et al., 2018). The presence of fiber content in whole grain flours and IHCF increases the density of extrudates, suggesting their suitability for the maximum dispersibility of flours and preparation of food products. Fiber typically causes the cell walls to rupture before the formation of bubbles, limiting the total expansion of the extruded product and developing a product with a higher density and less porous structure (Dos Santos et al., 2019).

3.2. Techno-functional characteristics

3.2.1. Water absorption index (WAI)

WAI indicates the ability to bind and hold water. It determines the acceptability of extrudates in terms of mouthfeel, juiciness, and texture. For breakfast cereals, high WAI is usually desirable. At the varied moisture content level from 12 % to 16 %, the WAI of the extrudates ranged from 6.73 to 7.76 g/g (Table 1). Increased WAI of extrudates may be related to the higher gelling capacity and increased number of available hydroxyl sites that bind with water molecules for better moisture penetration into the porous structure of extrudates (Machado Pereira et al., 2021). Also, the interaction between barrel temperature and higher feed moisture content revealed higher WAI values that enhance starch gelatinization, where the starch molecules are disrupted, and more water remains bound to the starch granule, leading to the improved WAI. A similar tendency was reported with replacing maize grit, gram flour, and rice grit with yam flour (KC et al., 2021).

3.2.2. Water solubility index (WSI)

WSI indicates the disruption of molecular components (Pardhi et al., 2019). The water solubility index measures the amount of polysaccharides released from the starch molecules in excess water after extrusion (Prabhakar et al., 2017). Reduced WSI values result in a low rate of starch degradation and less soluble molecules in the extrudates. The high water solubility index is an in vitro indicator of the digestibility of starch and the extent of its dextrinization and gelatinization (Guha et al., 1997). The highest WSI values were obtained for E-12, followed by E-13, E-14, E-15, and E-16 samples (Table 1), indicating more damage to starch granules. The main reason for higher WSI is the extrusion conditions, i.e., minimum feed moisture (12 %) and maximum barrel temperature (130 °C). An increase in feed moisture content in the formulation significantly decreased WSI, which is similar to previous literature on rice-based extrudates (Ding, Ainsworth, Tucker, & Marson, 2005) or maize-based extrudates (Kaur et al., 2022). A lower feed moisture content enhances the die's drag force and increases starch granules' shear disintegration, which can strengthen WSI (Sarawong et al., 2014).

3.3. Color analysis

Color is one of the key parameters for the quality index of extrudates that influences consumers' perception of the food product (Arivalagan et al., 2018). The color values of different extrudates are presented in Fig. 1. There was significant variation ($p \leq 0.05$) among CIELAB color coordinates of different samples treated with different feed moisture content. L^* values varied from 68.36 to 69.7; the value reduced (indicating darkening) as the conditioning moisture increased from 12 % to 16 %, whereas a^* (related to redness) and b^* (related to yellowness) values increased from 4.53 to 5.78 and 23 to 25, respectively with higher

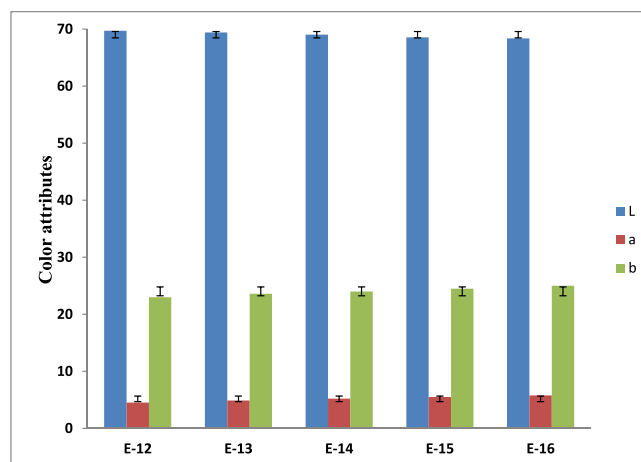


Fig. 1. L^* , a^* and b^* CIELAB color parameters of different extrudates. E-12 (extrudates with conditioning moisture of 12%), E-13 (extrudates with conditioning moisture of 13%), E-14 (extrudates with conditioning moisture of 14%), E-15 (extrudates with conditioning moisture of 15%), and E-16 (extrudates with conditioning moisture of 16%). Error bars represent standard deviation.

conditioning feed moisture. The decrease in lightness may be attributed to the increased moisture content, resulting in the formation of breakfast cereals with tightly packed air cells, which can increase light absorption and reduce luminosity (Promsakhaet et al., 2018). The pigment loss, i.e., Maillard reaction and caramelization, occurs during the extrusion process that changes the color of the ingredients (Zhang et al., 2020), leading to a reduction in L^* value and subsequent increase in a^* and b^* values (Jabeen et al., 2022).

3.4. Total phenolics and antioxidant activity

DPPH radical scavenging activity was used to evaluate the antioxidant activity of extruded products. The total phenolic content and DPPH radical scavenging activity of samples with varying conditioning moisture content are presented in Table 1. E-16 had the highest total phenolic and DPPH content among the tested samples, with a total phenolic and DPPH of 9.03 mg GAE/g and 30.36 %, respectively. The bioactive compounds are heat sensitive; thus, when barrel temperature (130 °C) and screw speed (380 rpm) were kept constant, total phenolic content and antioxidant activity were observed for all the extruded samples as the conditioning moisture content increased. The phenolic compounds might increase in the extrudates due to the hydrothermal process that disrupts the cell walls, releasing the bioactive compounds such as ferulic acid from the cell wall matrix and thus increasing total phenolic and antioxidant activity (Cheng et al., 2020). A similar increase in phenolic content and antioxidants was reported after extrusion cooking of raw banana flour enriched with defatted soy (Pandey et al., 2021) and buckwheat flour (Sun et al., 2018). However, a reverse trend was observed elsewhere as the phenolic acids probably undergo decarboxylation due to higher temperatures, and high feed moisture content also promotes polymerization, resulting in decreased efficiency of extractability of antioxidants and polyphenols (Ortiz-Cruz et al., 2020). As shown in Table 1, antioxidant activity showed a similar trend with total phenolic content. However, a higher retention rate of antioxidant activity was observed. Some previous research reported that melanoidin (Maillard products) produced during extrusion cooking can retain higher antioxidant content (Sharma et al., 2015), which could also support this study.

3.5. Microstructure: Scanning electron microscopy

The scanning electron micrographs for whole-grain flours (WWF,

WCF, WBF, and IHCF), their blend, and the extrudates at varying moisture levels are presented in Figs. 2 and 3. The SEMs for all the flours showed small and large starch granules and proteins cemented with the starch granules. The starch granules were round, polygonal, and irregular in shape; however, in WCF, the starch granules adhered less to the fibrous structures, which might be due to the less protein content. The granule sizes in WWF, WBF, WCF, and IHCF were 1.4–35 μm , with IHCF showing the smallest size of granules. In WWF, the starch granules were almost fully embedded in the protein matrix. The morphology of the granules depends on the biochemistry and physiology of the plant organelles, and the differences in shape and size are attributed to different plant origins (Svegmark and Hermansson, 1993).

The micrographs of the extrudates (Fig. 2) as a function of moisture were taken at 500 and 100 magnification levels. The extrudates showed dense inner structures with closed-air cells. Extrudates with higher moisture content showed fewer air cells formed during the expansion stage and, thus, higher hardness values. At lower feed moisture content, the formation of starch-lipid complexes is very high, which results in plasticization and more dense structures. This was evident from high enthalpies for extrudates with lower moisture content.

3.6. Differential scanning calorimetry

The thermal properties of processed flour, their blends, and modified flours are presented in Table 2. Raw flours and blends displayed one endothermic peak corresponding to starch gelatinization. Two endothermic peaks were recorded in the extruded flours: one for the starch gelatinization or protein denaturation and another peak called an amylose–lipid complex that indicates the “V” structure.

In extruded flours, at a constant temperature, screw speed, and increasing feed moisture content, the degree of gelatinization (G%) increased while gelatinization enthalpy (ΔH) and gelatinization

temperature range (T_c – T_o) reduced (Table 2). The gelatinization enthalpy indicates the loss of crystallinity. Thus, increasing feed moisture content reduced the crystalline order of whole-grain starches (Ji et al., 2004). The sample with the higher feed moisture content (E-16) indicates more starch gelatinization. Also, moisture content and high-temperature residual starch granules were more stable under increasing feed after extrusion.

The peak gelatinization temperature (T_p) represents the double helix length in crystalline quality (Hoover and Hadziyev, 1981). Increasing feed moisture content from 12 to 16 %, at constant barrel temperature and screw speed (130 °C and 380 rpm, respectively) significantly increased the T_p values. It decreased the gelatinization temperature range of extruded samples from 76.46 to 85.02 °C and 12.19 to 10.39 °C, respectively (Table 2). Endothermic transition with T_p at about 76.46 °C was observed for the E-12 sample having low moisture content. This peak would represent the retrograded starch molecule and the formation of the amylose–lipid complex after extrusion. At low moisture content with high temperatures, starch treatments showed greater homogeneity in the hydration, swelling, and fusion of starch crystals (Horndok and Noomhorm, 2007). Furthermore, the endothermic peaks of starch crystallites disappeared after extrusion, indicating the complete dissociation of starch crystallites during extrusion.

The onset gelatinization temperature (T_o) indicates the starch crystal perfection. Higher T_o represents more perfection of starch crystals. Thus, the starch crystallites to perfection, decreasing the onset of gelatinization temperature (Jafari et al., 2017).

Enthalpy of gelatinization measures the aggregation of the amylose–lipid complex. When the temperature is between melting and glass transition temperature, crystalline amylose–lipid complexes are formed. Increasing feed moisture content from 12 to 16 % decreased the gelatinization enthalpy. Water acts as a plasticizer during extrusion, and reducing feed moisture content enhances the melting and glass

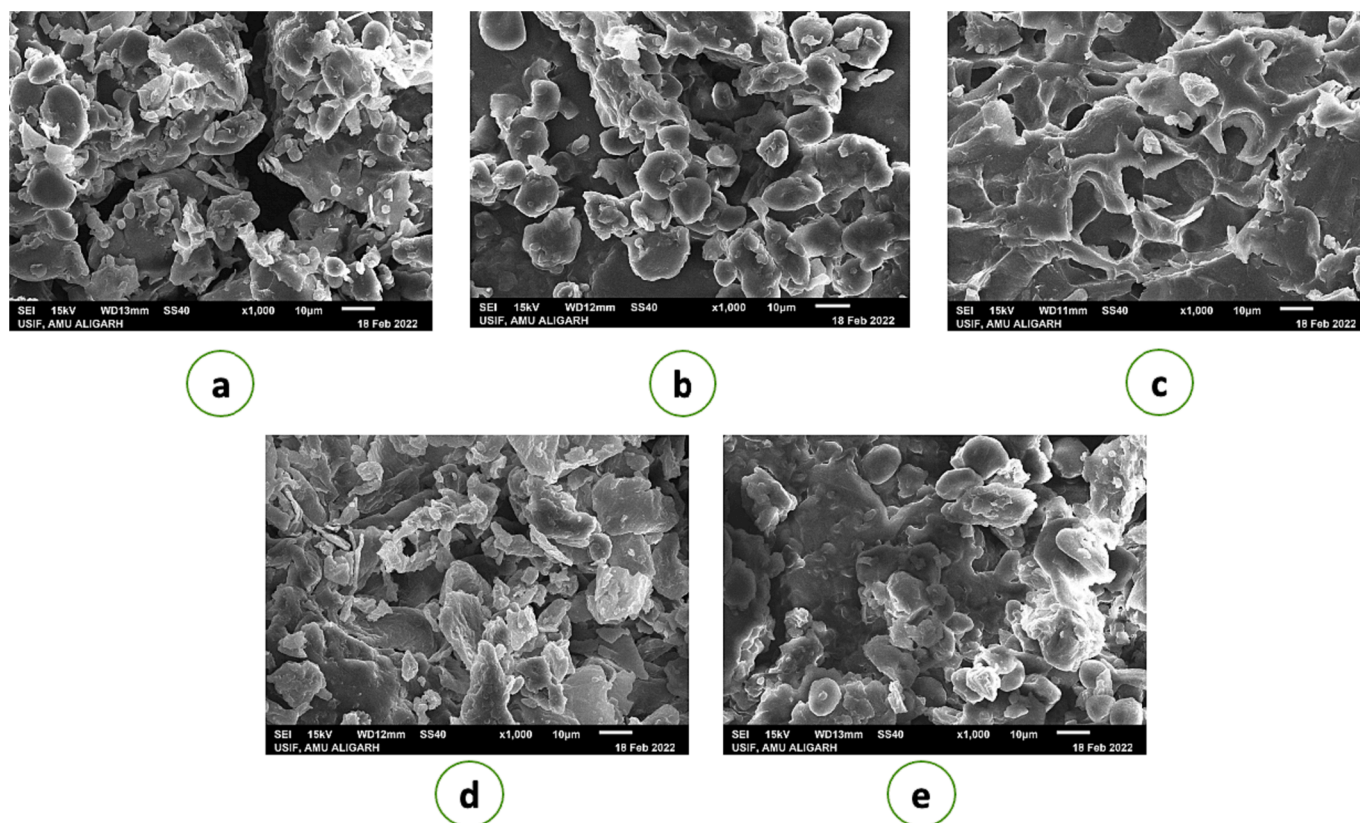


Fig. 2. Scanning electron micrographs of a) native whole wheat flour (WWF), b) whole corn flour (WCF), c) whole barley flour (WBF), d) Indian horse chestnut flour (IHCF) and e) their blend (10 % WWF, 10 % WBF, 2.5 % IHCF, 77.5 % WCF).

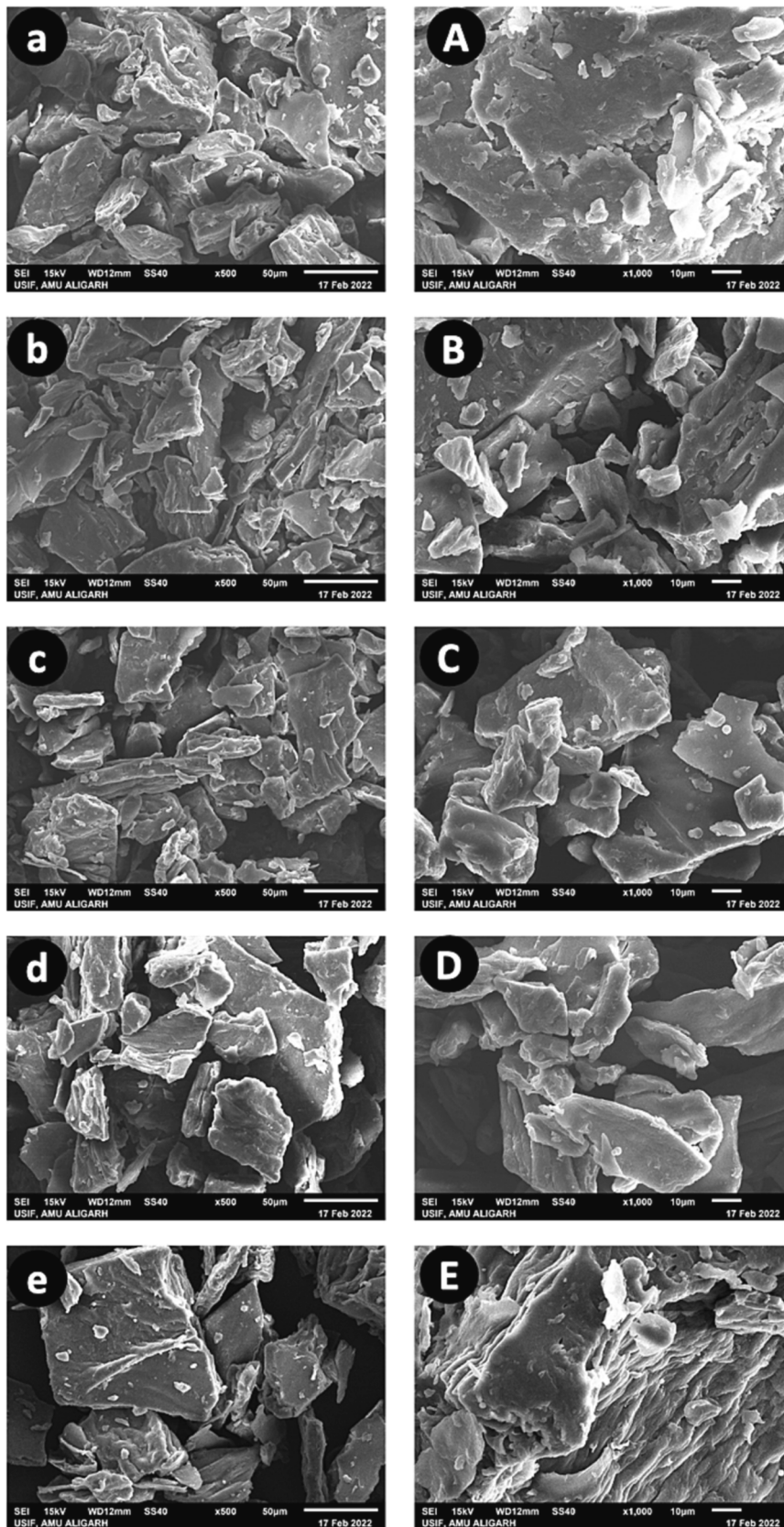


Fig. 3. Scanning electron micrographs of extruded flour at varying level of conditioning moisture content of a) 12, b) 13, c) 14, d) 15 and e) 16%. Capital letters represent micrographs of the same sample as that of the small letter but at a magnification of 1000X.

Table 2

Thermal and starch gelatinization characteristics of native and extrudates produced with varying levels of conditioning moisture.

Samples	T _o (°C)	T _p (°C)	T _c (°C)	ΔT (°C)	ΔH	G%
Native Flours						
WCF	68.52 ± 0.08 ^f	75.13 ± 0.13 ^f	80.25 ± 0.22 ^h	11.73 ± 0.03 ^g	9.23 ± 0.11 ^b	–
WWF	59.67 ± 0.11 ^h	65.30 ± 0.43 ^h	72.50 ± 0.15 ⁱ	12.83 ± 0.09 ^e	8.04 ± 0.04 ^d	–
WBF	64.20 ± 0.22 ^g	69.50 ± 0.04 ^g	82.60 ± 0.11 ^f	18.40 ± 0.22 ^a	7.90 ± 0.06 ^e	–
IHCF	55.50 ± 0.04 ⁱ	60.18 ± 0.07 ⁱ	65.72 ± 0.18 ^j	10.22 ± 0.14 ^j	9.76 ± 0.13 ^a	–
Blend	68.72 ± 0.09 ^f	75.98 ± 0.13 ^f	82.31 ± 0.11 ^g	13.59 ± 0.1 ^b	8.46 ± 0.11 ^c	–
Extrudates						
E-12	72.31 ± 0.17 ^e	76.46 ± 0.05 ^e	84.50 ± 0.02 ^e	12.19 ± 0.23 ^d	3.60 ± 0.16 ^f	57.44 ± 0.15 ^e
E-13	73.22 ± 0.1 ^d	77.97 ± 0.11 ^d	85.23 ± 0.11 ^d	12.01 ± 0.06 ^e	3.0 ± 0.04 ^g	64.50 ± 0.09 ^d
E-14	75.18 ± 0.05 ^c	79.38 ± 0.04 ^c	87.0 ± 0.17 ^c	11.82 ± 0.22 ^f	2.85 ± 0.23 ^h	66.30 ± 0.23 ^c
E-15	77.96 ± 0.03 ^b	80.94 ± 0.23 ^b	89.30 ± 0.1 ^b	11.34 ± 0.2 ^h	2.32 ± 0.08 ⁱ	72.57 ± 0.08 ^b
E-16	80.33 ± 0.21 ^a	85.02 ± 0.22 ^a	90.72 ± 0.13 ^a	10.39 ± 0.04 ⁱ	1.87 ± 0.13 ^j	77.89 ± 0.22 ^a

WCF = whole corn flour; WWF = whole wheat flour; WBF = whole barley flour; IHCF = Indian horse chestnut flour; Blend: 10 % WWF, 10 % WBF, 2.5 % IHCF, 77.5 % WCF; E-12 (extrudates with conditioning moisture of 12 %), E-13 (extrudates with conditioning moisture of 13 %), E-14 (extrudates with conditioning moisture of 14 %), E-15 (extrudates with conditioning moisture of 15 %), and E-16 (extrudates with conditioning moisture of 16 %). T_o = onset temperature; T_p = peak temperature; T_c = conclusion temperature; ΔT = gelatinization temperature range; ΔH = enthalpy of gelatinization; G (%) = degree of gelatinization.

Reported values correspond to the mean ± standard deviation. Different letters in the same column indicate significant differences (P < 0.05).

transition temperature (De pilli et al., 2008). Also, fiber plays a vital role as it restricts water availability and subsequently restricts starch gelatinization, reducing enthalpy. Generally, during extrusion, molecular structure distortion, and maximum starch gelatinization must have occurred that could release the amylose and bind with lipids to form more stable amylose–lipid complexes.

3.7. XRD analysis

X-ray diffraction has been extensively used to study the starch shape, type, degree of melting, gelatinization, and retrogradation (Ghoshal, Shivhare, & Banerjee, 2016). Different sources show different peak intensities and their precise diffractogram. The native whole grain flours i. e., WWF, WBF, and WCF, and their blend (10 % WWF, 10 % WBF, 2.5 % IHCF, and 77.5 % WCF) showed intensity peaks at 15.1°, 17.9° and 23°, indicating A-type crystalline nature (Kim et al., 2018) as shown in Fig. 4. In contrast, native IHCF showed C-type crystalline behavior with intensity peaks at 5°, 15.1°, 17.9°, and 27° (Rafiq, Singh, & Saxena, 2016). Compared to native flours, flour extruded at constant temperature (130 °C) and screw speed (380 rpm) but at varying moisture content (12 % to 16 %) exhibited diffraction peaks at 8°, 13.7°, 18.2°, and 20° indicating the V-type polymorph (Zhang et al., 2018; Cueto et al., 2018); peak at 20° being the main peak in all formulations. Oliveira et al. (2017) reported that the crystalline structure of unprocessed flour was altered and disrupted by the extrusion cooking process. During extrusion, loss of crystallinity occurs due to the disintegration of molecular bonds by intense mechanical shear force inside the extruder, leading to diffused amorphous materials (Shaikh, Ali, Mustafa, & Hasnain, 2020). Thus, low conditioning moisture content results in a higher expansion rate, less gelatinization, and simultaneous fragmentation (Lai, & Kokini, 1991). The V-type structure begins from amylose–lipid complexes,

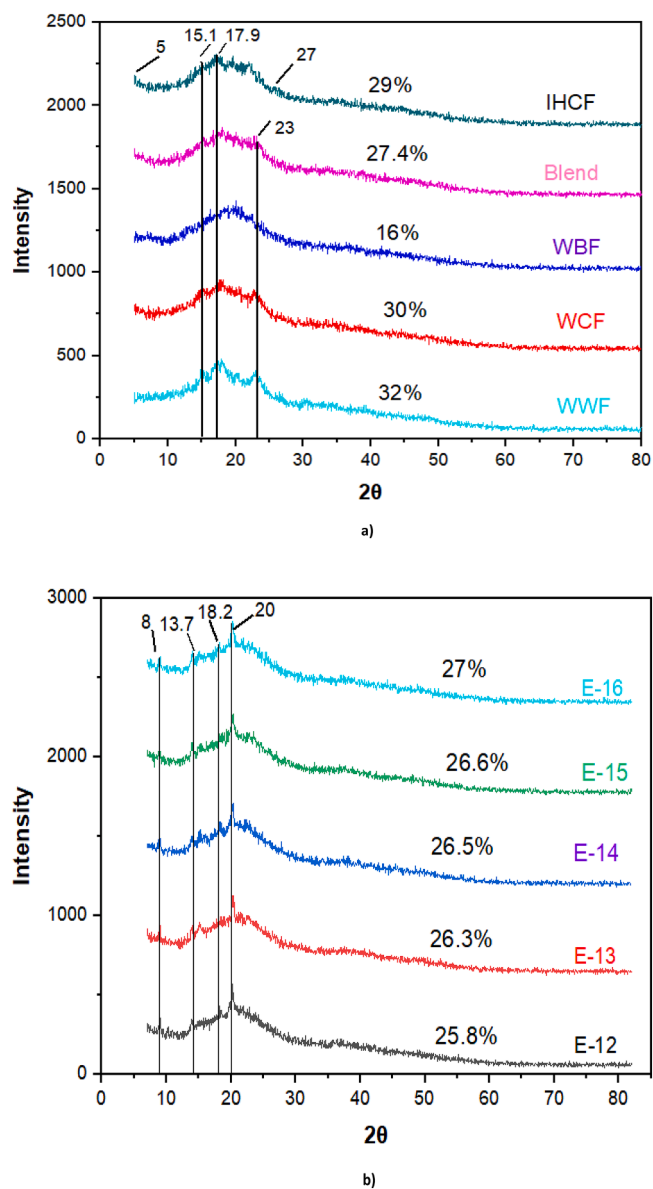


Fig. 4. X-ray diffractograms of a) native whole wheat flour (WWF), whole corn flour (WCF), whole barley flour (WBF), Indian horse chestnut flour (IHCF) and their blend (10 % WWF, 10 % WBF, 2.5 % IHCF, 77.5 % WCF) b) extruded flour at varying level of conditioning moisture content of 12, 13, 14, 15 and 16 %.

which are hardly found in native starch and are mainly formed after starch gelatinization during extrusion cooking (Cueto et al., 2018).

The relative crystallinity determined for native flours, their blend, and extruded products were also shown in Fig. 4. All the extruded flours showed a higher amorphous structure than native flours as the crystallinity reduced, representing starch disintegration during extrusion (Lopart et al., 2014). The degree of crystallinity of unprocessed flours and their blend was 32 %, 30 %, 16 %, 29 %, and 27.4 %, respectively (Fig. 4, a), and the crystallinity of extruded products varied from 27 % to 25.8 % (Fig. 4, b). Reducing the conditioning moisture content at high die temperatures negatively affects the crystallinity of extruded products (Fig. 4, b). The crystallinity of extruded flours reduced non-significantly as the conditioning moisture content decreased at constant temperature and screw speed. This might be due to the reduced flow rate of material, leading to enhanced shear force and residence time that would promote the degree of gelatinization of starch granules, as supported by the previous literature (Cheng et al., 2020).

4. Conclusion

This research reports the development of fiber-rich breakfast cereal from the blend of whole grain flour and non-conventional seed flour that has not been reported before. As the conditioning moisture content, mechanical shearing, and barrel temperature are high, starch is gelatinized more thoroughly to enhance the physico-functional and bioactive properties of extrudates. The SEM images showed that the gelatinization degree disrupts the crystalline nature of starch and produces a highly amorphous structure. The puffing of extruded products is the most desirable characteristic that significantly improves bulk density, sectional expansion, and water absorption capacity. bulk whole-grain The degree of gelatinization (%), peak gelatinization temperature (T_p), and starch crystallinity (%) showed a positive correlation with the feed moisture content. The extrusion cooking process alters the A-type structure of unprocessed starch to V-type pattern with main intensity peak at 20°. Reducing feed moisture content negatively affects the starch crystallinity. Thus, the blend of whole grain flours with non-conventional seed flour and the selection of suitable process conditions could potentially be utilized at an industrial level for the development of breakfast cereals from fiber-rich ingredients.

CRedit authorship contribution statement

Farhana Mehraj Allai: Conceptualization, Methodology, Writing – original draft, Formal analysis. **Pir Mohammad Junaid:** Writing – original draft, Formal analysis. **Z.R.A.A. Azad:** Conceptualization, Methodology, Supervision, Writing – review & editing, Supervision. **Khalid Gul:** Conceptualization, Writing – review & editing, Supervision. **B.N. Dar:** Conceptualization, Writing – review & editing, Supervision. **Shahida Anusha Siddiqui:** Writing – review & editing, Formal analysis. **Jose Manuel Loenzo:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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