Food Measure (2017) 11:1284–1294
DOI 10.1007/s11694-017-9506-5

ORIGINAL PAPER

# Nutritional, functional, phytochemical and structural characterization of gluten-free flours

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Received: 8 September 2016 / Accepted: 27 February 2017 / Published online: 11 March 2017


**Abstract** Different gluten-free flours from various sources may vary in composition and properties, thereby affecting applications in food systems. Various gluten-free flours viz. sorghum, rice, moong, water chestnut flour (WCF) and unripe banana flour (UBF) were studied in comparison with wheat flour. These were studied for their proximate composition, pH, iodine affinity value, leaching, functional properties, phytochemical analysis, color analysis, and X-ray diffraction analysis. Protein content in these flours ranged from 5.31 to 24.07%moong flour having highest protein content. Variations were observed with fat percentage from 1.01 to 3.45%. All flours were high in carbohydrate content i.e. ranged between 61.63 to 81.19%. pH values indicated slight acidic nature of all flours. UBF was found to have maximum iodine affinity value (46086 ppm). Leaching study indicated the extent of leaching with respect to time in these flours. Light transmittance was decreased with the duration of cooking for all flours. Color analysis values showed the difference in the L*, a, b values. X-ray diffractograms revealed the crystallinity patterns of the flours with percentage crystallinity which ranged from 22.6 to 34.9.This study concludes that wheat can be substituted by these flours in various applications.

**Keywords** Gluten-free · Moong · Phytochemicals · Unripe banana · Water chestnut

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## Introduction

The growing number of people diagnosed with Celiac Disease, together with a general demand for novel tasty and healthy foods, has given birth to a new market consisting of cereal products made from grains alternative to wheat. Bread being most abundantly consumed stands in a position of global importance in human nutrition [1]. Rajpoot and Makharia [2] revealed in their study that 6–8 million Indians are estimated to have celiac disease although only fraction of them have been diagnosed. They reported; while the number of patients with celiac disease is increasing, the country's preparedness towards the emerging epidemic of this disease is minimal. Industrial production of reliable and affordable gluten-free food is one of the key issues requiring urgent attention.

Currently, number of studies is emphasizing the need for an improvement in the nutritional quality of cereal based gluten-free products. The demand for high-quality gluten-free (GF) bread is increasing which represents a challenging task for the food technologist due to the low baking quality of GF flours as a consequence of the absence of gluten network. Hence, recent studies focus on new technologies as tools to improve the bread making performance of GF grains.

Commercial GF breads are mainly starch-based and therefore lack fiber, vitamins and nutrients, which causes worsening effect on the already nutritionally unbalanced diet of celiac disease sufferers who strictly are adhered to a GF diet [3]. Furthermore, these breads possess inferior quality, present deprived crumb and crust characteristics, rapid staling, along with poor mouth feel and flavor [4]. This necessitates the need for high-quality GF foods [5].

Various reports are available on the chemical composition of cereal grains, but data on the composition of gluten-freeflours is scarce.

Many wheat flours substitutes have been applied to produce GF Bread, being the most common rice flour, corn flour, potato starch, millet [*Pennisetumglaucum*(L.) R.Br.], quinoa (*Chenopodium quinoa* Wild), amaranth (*Amaranthus*spp. L.), buckwheat (*Fagopyrumesculentum* Moench.), soya [*Glycine max* (L.) Merr.], chickpea (*Cicerarietinum*L.), cassava (*Manihotesculenta* Crantz.) and,in a lower scale, sorghum flour [6].

Perhaps, rice flour (*Oryzasativa*) is the most commonly used gluten-free flour in industry as well as for research purposes. Rice flour is an economical and easily accessible nutrient source. Sorghum has been neglected over the past decades and also doesn't play an important role in commercialized food systems today. There are limited research efforts in grain processing and product technologies to assess the potential of this crop for food uses [7]. Moong bean (Vignaradiata (L.) or green gram is inherent to the northeastern India–Burma (Myanmar) region of Asia. It is predominantly grown for its protein-rich edible seeds. Moong bean is similar in composition to other members of the legume family, with 24% protein, 1% fat, 63% carbohydrate and 16% dietary fiber (US Department of Agriculture, 2001). Water chestnut commonly known as Singhara (Trapabispinosa Roxb.) in Indian subcontinent can is an underutilized tuber is eaten raw, boiled or roasted, and the excess production of the fruit is preserved by drying (sun or mechanical drying) followed by ground into flour for commercial uses. Water chestnut flour (WCF) is an admirable source of energy owing to its high starch content. The fruit contains nutritionally rich compounds such as omega-3 fatty acids, vitamins and antioxidative agents such as phenolic and tannins [8].

According to the FAO, banana (Musa spp.) is amongst the world's prominent crops, after rice, wheat and maize. The worldwide production of banana tends to increase and was 102 million tonnes in 2010. Over 130 countries produce banana. Ecuador, Colombia, Philippines and Costa Rica are the leading exporters (FAOSTAT, 2011). When banana is green or unripe, it is very rich in indigestible carbohydrates (up to 60–80%), which is composed of cellulose, hemicelluloses, lignin, starch, dietary fiber, and resistant starch (RS2) [9–12]. Till date, use of banana flour has not gain popularity in food industry despite of its nutritional advantages.

While the preparation of any food, ingredients are considered mainly for their physical and functional properties which will impart the final products with desirable qualities and simplify processing of the products. The aim of this preliminary study was to characterise the chemical composition of gluten-free flours made from sorghum, rice, moong (MF), water chestnut (WCF) and unripe banana (UBF) and to compare their properties to that of wheat flour. The information gained in this research is crucial for the formulation of gluten-free products with improved quality such as bread and pasta.

## Materials and methods

### Materials

Wheat (*Sharbati* variety), rice (*Indrayani* variety), sorghum (*Kharip* variety), water chestnut (Indian variety) and moong flour (*Kharip* variety) were purchased from the local market of Mumbai, Maharashtra. Unripe banana flour (*Basrai* variety) was procured from Jalgaon banana market,Maharashtra. All the flours were sieved (60 mesh) and then used for analysis.

### Proximate composition

*M*oisture and ash contents were determined by AOAC (1995) methods [13]. Protein content determined by AACC (2000) methods [14], whereas fat content was determined by AOAC (2006) method [15]. Carbohydrate content was calculated by difference.

### Physicochemical properties

For determination of bulk and tapped densities; method used by Olorunsola et al. [16] was adopted. Carr's Index and Hausner's rationwere determined as explained by Aulton (1996). These are frequently used as indication of the flowability of a powder [17]. Water and oil absorption determinations were carried out as described by Abbey and Ibeh [18] using 1 g flour and 10 ml distilled water or olive oil. The color of all samples was measured by using Hunter Lab Color Quest. (Labscan XE). L value indicates degree of lightness or darkness; a and b values indicate degree of redness or greenness and yellowness or blueness, respectively [19, 20].

Leaching of flours was studied by the method elucidated by Morawicki et al. [21]. The iodine affinity was assayed using guidelines of Kawabata et al. [22].

### Extraction

Flour sample (1 g) was extracted for 3 h with 10 ml of methanol-HCL solvent on an orbital shaker set at 180 rpm (30 ± 1 °C). The extract obtained after shaking was further centrifuged at 10,000 rpm at 37 °C. The supernatant was collected and stored at 4 ± 1 °C in amber colored bottles until further analysis. The analysis was carried out within 3 days.

### Phytochemical Analysis

The total phenolic content was measured by Folin–ciocalteau method explained by Singh et al. [23]. 0.2 ml extract was used for the analysis. The standard curve was linear between 0 and 100 µg/mL gallic acid. Results were represented as mg of GAE/g of flour.

Flavonoid content was determined by the method explained by Qin et al. [24] using 0.5 ml extract. The standard curve was linear between 0 and 100 µg/ml quercetin. Results were expressed as mg of QE/g of flour.

Tannin content was evaluated by the method of Sharma et al. [25]. The standard curve was linear between 0 and 100 µg/ml tannic acid.

### X ray diffraction

X-ray diffraction measurements were performed on 1 g samples of flours which were packed tightly in rectangular silicon cells. X-ray diffraction patterns were obtained with a diffractometer (RigakuMiniflex) using monochromatic Cu-Ka radiation of 1.5406 A°. The diffractometer was operated at 30 kV, 15 mA and 123 spectra scanned over a diffraction angle (2h) range of 2–40 with scanning rate of 3/min as explained by Sonawane et al. [26].

### Statistical analysis

Results were expressed as the mean values of three replications ± standard deviation. The results were submitted to variance analysis (ANOVA) using the SPSS 17.0 statistical software programme (SPSS Inc., Chicago, IL, USA) and the means were compared using the Duncan's post hoc test and $p < 0.05$ were regarded as significant. Principal component analysis (PCA), which is a multivariate approach designed for multi-correlated data was done using STATISTICA 7.

## Result and discussion

### Proximate composition

The proximate composition of wheat flour and gluten-free flours analyzed is presented in Table 1. All the flours including wheat and non-wheat had optimum moisture content (Table 1), similar to those determined in commercial dry products such as wheat germ, oat cookies and bran flakes [27]. Among the samples, MF has the highest protein content (24.07%) followed by wheat (11.13%) and sorghum (10.2%). Gopalan et al. [28] also found 24 g of protein per 100 g green gram flour. Similar results were reported by Ahmed et al. [29] mentioning 8.4% protein content for water chestnut flour. Fat content of flours ranged from 1.01% for UBF to 3.45% for sorghum. Hager et al. [30] have reported similar levels of fat content in sorghum. Nimsung et al. [31] while studying various banana cultivars found to contain fat from 1.56 to 4.88%. Ash content of flours was found to be 1.42% for wheat and ranged between 1.2 to 2.92% for non-wheat flours. The carbohydrate content of sorghum (74.49%) and rice (77.33%) was comparable to wheat (75.21%) while WCF, UBF possessed higher carbohydrate content (80.49, 81.19% respectively) and MF had less percentage of carbohydrates (61.63%) when compared to wheat. Rahman and Uddin [32] have reported total carbohydrate of MF flour as 63.4% which is in agreement of our observations. Gani et al. [33] and Bezerra et al. [34] have stated comparable values for WCF and UBF respectively.

**Table 1** Proximate composition of wheat and gluten free flours

| | Moisture (%) | Protein (%) | Fat (%) | Ash (%) | Carbohydrate by difference (%) |
|---|---|---|---|---|---|
| Wheat | $9.86 \pm 0.94^{a}$ | $11.13 \pm 0.15^{d}$ | $2.36 \pm 0.32^{c}$ | $1.43 \pm 0.20^{a}$ | $75.21 \pm 1.28^{bc}$ |
| Sorghum | $9.41 \pm 1.10^{a}$ | $10.2 \pm 0.36^{c}$ | $3.45 \pm 0.36^{d}$ | $2.43 \pm 0.19^{bc}$ | $74.49 \pm 0.96^{b}$ |
| Rice | $11.54 \pm 0.75^{a}$ | $7.7 \pm 0.3^{b}$ | $2.21 \pm 0.22^{c}$ | $1.20 \pm 0.11^{a}$ | $77.33 \pm 1.18^{c}$ |
| MF | $9.74 \pm 1.01^{a}$ | $24.07 \pm 0.69^{e}$ | $1.61 \pm 0.35^{b}$ | $2.92 \pm 0.44^{d}$ | $61.63 \pm 1.28^{a}$ |
| WCF | $9.30 \pm 0.75^{a}$ | $5.57 \pm 0.33^{a}$ | $1.94 \pm 0.17^{bc}$ | $2.68 \pm 0.25^{cd}$ | $80.49 \pm 0.74^{d}$ |
| UBF | $10.26 \pm 1.98^{a}$ | $5.31 \pm 0.29^{a}$ | $1.01 \pm 0.11^{a}$ | $2.22 \pm 0.11^{b}$ | $81.19 \pm 1.99^{d}$ |

Values are represented as means ± SD of triplicate readings

Subsets with a common letter in a column are not significant are not significant at 5% probability level

*MF* moong flour, *WCF* water chestnut flour, *UBF* unripe banana flour

## Functional properties

Functional properties have been categorized as the non-nutritive characters that food constituents play in a food system. Functionality of flour is important in the preparation, processing, storage, quality, and sensory attributes of foods. Knowledge of functional property is critical for the development of new products and the improvement of existing one.

The bulk densities of the samples range between 0.45 and 0.66 g/cm$^3$ while the tapped densities varied from 1.32 to 1.76 g/cm$^3$ as shown in Table 2. The difference between the bulk and tapped density was higher in case of sorghum which signifies that the volume of the flour will decrease excessively during storage or distribution. Compressibility describes the cushioning ability of a material and is related to its relative softness or hardness. Low bulk compressibility is desirable for loose fill packaging material [35].The tapped densities of rice, MF, WCF and UBF were found low compared to wheat flour (1.33, 1.44, 1.32 and 1.36 g/cm$^3$ respectively) thereby making these flours suitable for the formulation of high nutrient density weaning food [36].

The Carr's index and Hausner's ratio are the micromeritic properties of a powder which determines the flow characteristics and compressibility of a powder. The Carr's index and Hausner's ratio above 23% and 1.2 respectively do not indicate good flow and compressibility properties [37]. According to the statement presented by Samborska et al. [38], on the grounds of the results shown in Table 2 it was alleged that all the samples including wheat and non-wheat flours were characterized by high cohesiveness and poor flowability.

The water absorption capacity of wheat flour was 363.68% whereas for MF showed the highest water absorption capacity 428.16%. The extent of protein hydration correlates strongly with the content of polar residues as well as the interaction between water molecules and hydrophilic groups which occurs via hydrogen bonding. The higher protein content of MF might be responsible for high hydrogen bonding and high electrostatic repulsion, both conditions facilitating binding and entrapment of water [39] which tends to increase water absorption. Higher water absorption capacities in case of WCF and UBF could be attributed to the presence of higher amount of carbohydrates (starch) and fiber in this flour. Chandra and Samsher [40] also found the highest water absorption capacity for potato flour due to higher starch content. Hence, higher water absorption capacity of these gluten-free flours gave them an advantage of being used as a thickener in liquid and semi-liquids foods since the flours has the ability to absorb water and swell for improved consistency in food. Water absorption capacity is a critical function of protein in various food products like soups, dough and baked products [41].

The highest oil absorption capacity was observed for sorghum (202.8) and UBF (202.22%) followed by WCF (193.96%), rice (191.51%), and MF (190.87) and then wheat (186.28). The water and oil binding capacity of food protein depends upon the intrinsic factors like amino acid composition, protein conformation and surface polarity or hydrophobicity [40]. The ability of the proteins of these flours to bind with oil makes it useful in food system where optimum oil absorption is desired. The oil absorption capacity also makes the flour suitable in facilitating enhancement in flavor and mouth feel when used in food preparation. Due to these properties, the protein probably could be used as functional ingredient in foods such as whipped toppings, sausages, sponge cakes etc.

pH value is one of the physico-chemical property important for application, being used to indicate the acidic and alkaline property of the sample. pH values for the flour samples ranged from 5.06 to 6.12 (Table 2), this shows that the flours contain low acid content. It may be due to presence of free fatty acids in these flours [42].

**Table 2** Physico-functional properties of wheat and gluten free flours

| | Bulk density (g/cm$^3$) | Tapped density (g/cm$^3$) | Carr's index (%) | Hausner's ratio | Water absorption capacity (%) | Oil absorption capacity (%) | pH | Iodine affinity value (ppm) |
|---|---|---|---|---|---|---|---|---|
| Wheat | 0.52 ± 0.01$^a$ | 1.49 ± 0.01$^d$ | 65.41 ± 0.58 $^{cd}$ | 2.89 ± 0.05$^c$ | 363.68 ± 5.96$^b$ | 186.28 ± 8.27$^a$ | 5.76 | 26086.96 ± 0$^b$ |
| Sorghum | 0.46 ± 0.01$^b$ | 1.76 ± 0.01$^e$ | 73.66 ± 0.33$^e$ | 3.79 ± 0.05$^e$ | 415.84 ± 5.39$^e$ | 202.80 ± 10.44$^a$ | 5.84 | 24347.83 ± 1506.13$^{ab}$ |
| Rice | 0.66 ± 0.02$^d$ | 1.33 ± 0.02$^a$ | 50.03 ± 2.72$^a$ | 2.01 ± 0.11$^a$ | 415.54 ± 7.03$^d$ | 191.51 ± 6.10$^a$ | 5.66 | 19130.43 ± 3012.26$^a$ |
| MF | 0.51 ± 0.01$^b$ | 1.44 ± 0.01$^c$ | 64.56 ± 0.27$^c$ | 2.82 ± 0.22$^c$ | 428.16 ± 7.70$^a$ | 190.87 ± 10.12$^a$ | 6.12 | N.D |
| WCF | 0.57 ± 0.01$^c$ | 1.32 ± 0.01$^a$ | 56.58 ± 0.74$^b$ | 2.30 ± 0.04$^b$ | 387.33 ± 7.68$^c$ | 193.96 ± 10.45$^a$ | 5.96 | 26956.22 ± 1506.13$^b$ |
| UBF | 0.45 ± 0.01$^a$ | 1.36 ± 0.11$^b$ | 66.83 ± 0.39$^d$ | 3.01 ± 0.04$^d$ | 385.28 ± 1.77$^c$ | 202.22 ± 8.01$^a$ | 5.06 | 46086.96 ± $^c$ |

Values are represented as means ± SD of triplicate readings

Subsets with a common letter in a column are not significant are not significant at 5% probability level

*MF* moong flour, *WCF* water chestnut flour, *UBF* unripe banana flour

Amongst all the flours UBF showed highest iodine affinity value (46,086 ppm) i.e. contains more amylose [43–46] reflecting the capability of the amylose to form a complex with iodine. WCF has the similar iodine affinity value as that of wheat while flour of Sorghum and rice showed low affinity to iodine as compared to wheat. Amon et al. [47] suggested that high iodine affinity of starch exhibit moderate or lower paste clarity.

### Leaching of solids during cooking

Figure 1 illustrates the leaching behavior of flour samples plotted as transmittance as a function of cooking time. It clearly shows the decrease in transmittance as the duration of cooking increased. Morawicki et al. [21] stated that the transmittance correlates fairly with the solids leached while studying rice cooking. As depicted in Fig. 1, rice had the highest paste clarity in contrast with wheat and other samples. Paste clarity is another important property of flour or starch that governs which applications different flours or starches may have for food processing. The results presented here indicate differences in paste clarity, which may determine which kind of flour, or starch may be used for different applications in the food industry. There are many factors that may also influence paste clarity such as amylose, lipid and protein contents [48], botanical source, and particle size of granules, total solids concentration, degree of granule dispersion, and the capacity of granules to form aggregates [49–51], which have not been examined to any great extent in this study. However the results here indicate gluten-free flours may offer high enough paste clarity for use in food products requiring this.

### Color properties

Table 3 elaborates the color values of gluten-free flours compared to wheat. The color of the flour due to the presence of polyphenolic compounds, ascorbic acid and carotene has impact on its quality. Any pigmentation in starch is carried over to the final product. This reduces the quality, hence acceptability of starch product [52]. Positive L value closer to 100 suggests the white color of the flour. Rice flour possesses highest L value (93.65) while lower L value in case of UBF (76.35) was due to the dark color of the flour. L* reduction is due to the pigments produced during the polyphenol oxidase–mediated browning reaction upon the phenolic compounds present in the UBF [53] which may be due to the enzymatic browning during traditional drying. Negative 'a' value for rice reveals the presence of green pigments while a positive value signifies red color. However, for all the samples including wheat this value is comparatively smaller compared to 'L' and 'b' which signals the negligible extent of these colors. 'b' value (yellowness)was the highest in case of

**Table 3** Color parameters of wheat and gluten free flours

| No. | Sample | L value | a value | b value |
|---|---|---|---|---|
| 1 | Wheat | $85.29\pm0.01^{c}$ | $1.96\pm0.02^{e}$ | $13.26\pm0.26^{e}$ |
| 2 | Rice | $93.66\pm0.02^{g}$ | $-0.043\pm0.03^{a}$ | $5.12\pm0.02^{b}$ |
| 3 | Sorghum | $86.58\pm0.01^{d}$ | $1.07\pm0.03^{d}$ | $1.84\pm0.02^{a}$ |
| 4 | MF | $89.89\pm0.02^{f}$ | $0.049\pm0.02^{b}$ | $20.24\pm0.02^{g}$ |
| 5 | WCF | $82.53\pm0.02^{b}$ | $2.09\pm0.02^{f}$ | $12.58\pm0.02^{d}$ |
| 6 | UBF | $76.35\pm0.02^{a}$ | $3.22\pm0.02^{g}$ | $14.26\pm0.02^{f}$ |

Values are represented as means ± SD of triplicate readings

Subsets with a common letter in a column are not significant are not significant at 5% probability level

*MF* moong flour, *WCF* water chestnut flour, *UBF* unripe banana flour

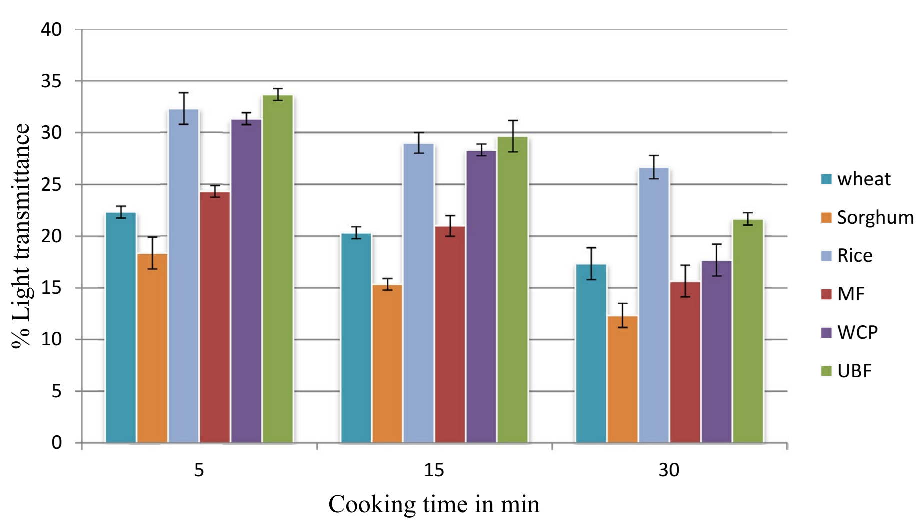


**Fig. 1** Leaching behaviour of flours. MF : Moong flour, WCF: Water chestnut flour, UBF: Unripe banana flour

MF which could be due to the carotene content of the split green gram [54].

### Bioactive components

Phenolic compounds ubiquitous in plants are key phytochemical drivers of the health and functional foods and nutraceuticals industry. Research with polyphenol compounds from various crops has created a growing market for polyphenol-rich ingredients (Nutraingredients). Our present investigation depicts higher content of total phenolics in WCF and UBF (101.09 and 109.13 mgGAE/g respectively) than wheat flour (98.91mgGAE/g) as shown in Table 4. Whereas, Sorghum, rice and MF were lower in total phenolic content than wheat flour. Similar values of phenolic content were observed by Sakac et al. [55] for rice flour while developments of gluten free cookies. Hithamani and Srinivasan [56] studied the bioaccessibility of polyphenols and found total polyphenols compound in whole grains of wheat, sorghum and green gram to be 1.20, 1.12 and 4.03 mg/g respectively. Researchers found that the variations in total phenolic content in different plant sources are due to some non-phenolic reducing compounds, such as organic acids and sugars, which interferes the determination of total phenolic contents by the Folin–Ciocalteu method, which leads to an overvaluation of the phenolic content [57]. Furthermore, different phenolic might present different responses with the Folin Ciocalteu reagent.

Flavonoids as one of the most diverse and wide spread group of natural compounds are probably the most important natural phenolic [58].The content of total flavonoids in flours ranged from 13.81 to 34.32mgQE/g as given in Table 4. WCP and UBF were better sources for flavonoids than wheat and other flour samples. Hithamani and Srinivasan [55] found the extent of flavonoids in wheat, sorghum and green gram to be 0.84, 0.89 and 1.56 mg/g correspondingly. Differences in the values observed may be due to the varieties, variation in soil and environmental conditions etc. Also, these variations could be attributed to the inherent variability of the raw material, as well as to the differences in methodology or standard used [59].

**Table 4** Bioactive constituents of wheat and gluten free flours

| | Total phenolic content (mgGAE/g) | Flavonoid content (mgQE/g) | Tannin content (ug TA/g) |
|---|---|---|---|
| Wheat | $98.91 \pm 2.39^{b}$ | $27.75 \pm 1.84^{c}$ | $290.27 \pm 18.78^{b}$ |
| Sorghum | $90.21 \pm 0.65^{a}$ | $26.04 \pm 2.29^{bc}$ | $282.39 \pm 9.97^{b}$ |
| Rice | $90.21 \pm 0.65^{a}$ | $13.81 \pm 2.92^{a}$ | $150.27 \pm 1.82^{a}$ |
| MF | $89.78 \pm 1.52^{a}$ | $24.22 \pm 1.14^{b}$ | $298.45 \pm 9.49^{b}$ |
| WCF | $101.09 \pm 1.95^{c}$ | $34.32 \pm 0.46^{d}$ | $510.58 \pm 11.43^{c}$ |
| UBF | $109.13 \pm 0.43^{b}$ | $34.12 \pm 1.04^{d}$ | $520.58 \pm 5.16^{c}$ |

Values are represented as means ± SD of triplicate readings

Subsets with a common letter in a column are not significant are not significant at 5% probability level

*MF* moong flour, *WCF* water chestnut flour, *UBF* unripe banana flour

Amongst all the flour samples studied, WCF and UBF (510.58 and 520.58 mgTA/100 g respectively) found to contain higher amount of tannins as compared to wheat (290.27 mgTA/100 g). Sorghum and MF (282.39 and 298.45mgTA/100 g)) had content of tannins with little difference to wheat whereas rice contained least amount of tannins (150.27 mg TA/100 g). Hithamani and Srinivasan [55] found the tannin content to be in the order 0.45, 0.62 and 4.05 mg/g for wheat, sorghum and green gram respectively.

The study of bioactive constituents showed their higher concentration in UBF and WCF compared to wheat.

### XRD Characteristics

There are three recognized types of starch crystallinity patterns, and these are commonly designated as A, B and C [60]. The assignment of a particular starch to one of the groups has generally been based on the direct comparison of the X-ray diffractograms with those of starches with established and characterized patterns. Figure 2 shows the diffractograms of wheat flour and gluten-free flours. Two well-defined peaks at 2 theta 15.1° and 23° were located in patterns from wheat, rice and sorghum. It shows the presence of A-type starch. The presence of A-type starch indicates that the semi-crystalline nature present in the fruit [61]. In case of UBF and WCP peak is positioned at 2 theta 15.1° in the pattern; however, the peak at 2 theta = 23° is not well defined. According to XRD pattern this flour contains B-type starch. These observations are in agreement with Hoover and Sosulski [62] who referred cereal starches being A-type whereas tuber starches to show B-type pattern. There have been fewer studies of legume starches, there have been reports that these are different again and have patterns having intermediate characteristics to the A- and B-types of starches [63–67]. The X-ray pattern of MF revealed C-type starch X-ray pattern. This was characterized by strong intensity peaks corresponding approximately to 15°, 17° and 23° 2θ. However, the C-type starches previously reported to occur in legumes, are generally more resistant to digestion and differ in their reflection intensity from the other types of starches [68]. Sarco and Wu [69] suggested that at the molecular level, the A-and B-type structure differ in the crystalline packing of the helices and water content, the B-type has much more space available for water than the A-type. This is in accordance with our data as shown in Table 5. WCF and UBF possess the higher spacing value (4.362 nm and 4.587 nm respectively) as compared to wheat (3.811 nm), rice (4.139 nm), sorghum

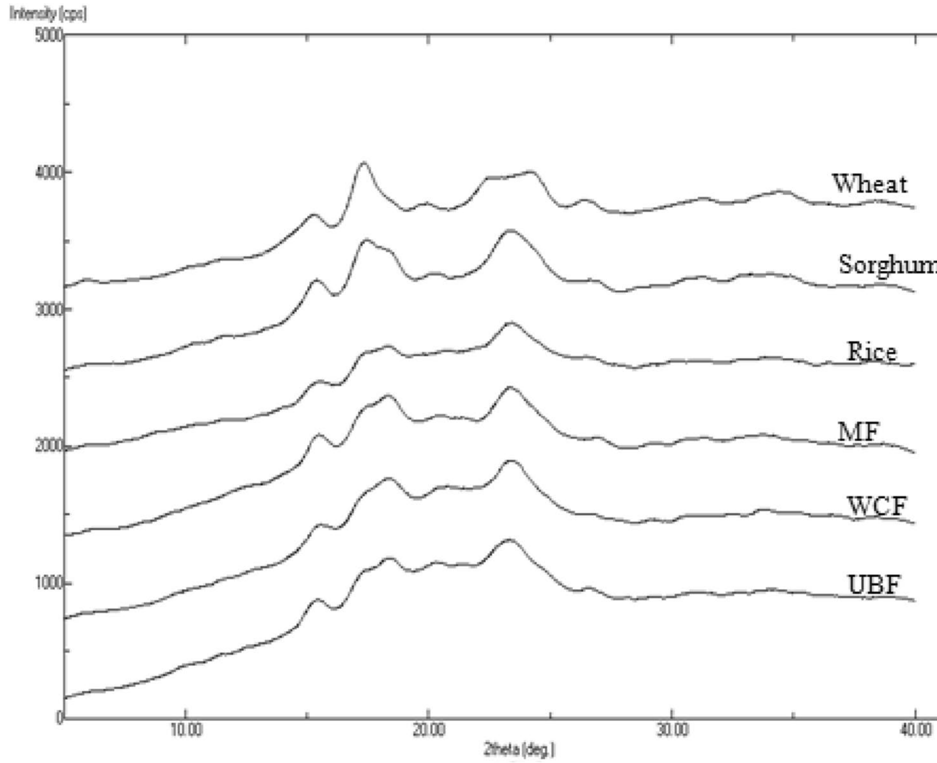


**Fig. 2** XRD diffractograms of wheat and gluten free flours. *MF* moong flour, *WCF* water chestnut flour, *UBF* unripe banana flour

**Table 5** XRD parameters of wheat and gluten free flours

| Sample | 2 theta | d value (nm) | % crystallinity |
|---|---|---|---|
| Wheat | 23.59 | 3.81 | 22.6 |
| Sorghum | 21.69 | 3.98 | 23.8 |
| Rice | 21.22 | 4.14 | 28.2 |
| MF | 23.55 | 4.25 | 22.8 |
| UBF | 17.52 | 4.36 | 34.9 |
| WCP | 16.39 | 4.59 | 34.9 |

Values are represented as means ± SD of triplicate readings

*MF* moong flour, *WCF* water chestnut flour, *UBF* unripe banana flour, d value: interplanar spacing)

(3.980 nm) and MF (4.249 nm). Ghasemi et al. [70] while studying water absorption behavior of wood flour noticed that higher d values are associated with stronger order of intercalation i.e. stronger insertion of a molecule or ion into the layers of solid.

### Principal component analysis

Principal Component Analysis (PCA) was used to visualize the variation between the characteristics of wheat flour from gluten-free flours [71]. With this statistical method, a large number of variables are reduced to a few variables called principal components (PCs) that describe the greatest variance in the data analyzed. The PCA plots provide an overview of the similarities and differences between the flours and of the interrelationships between the measured properties. The distance between the locations of any two flours on the score plot is directly proportional to the degree of difference or similarity between them. This analysis showed two axes explaining the essential variability that were axis 1 and 2. The first and the second PCs described 36.59 and 27.10% of the variance respectively. Together, the first two PCs represented 63.69% of the total variability. As shown in Fig. 3a, PC1 (principal component 1) separates sorghum, MF and rice from WCF and UBF. Sorghum and MF (characterized by high TD, HR and L values) are positively influenced by second principal component while rice (distinguished by high BD) has large negative score in PC2. Wheat (high 2 theta value) is not well explained by these two PCs. The plot suggests the close resemblance between MF and WCF to wheat as they are placed closer to wheat on the plot.

The loading plot of the two PCs provided the information about correlations between the measured properties (Fig. 3b). The properties whose curves lie close to each other on the plot were positively correlated while those whose curves run in opposite directions were negatively correlated. PC1 is well characterized by TFC, tannin, TPC, d value, carbohydrate, a value, TD, protein, pH, fat, WAC and L value. The second principal component links HR, CL, TD, Ash, b value, OAC, BD, moisture to sorghum, MF and rice.

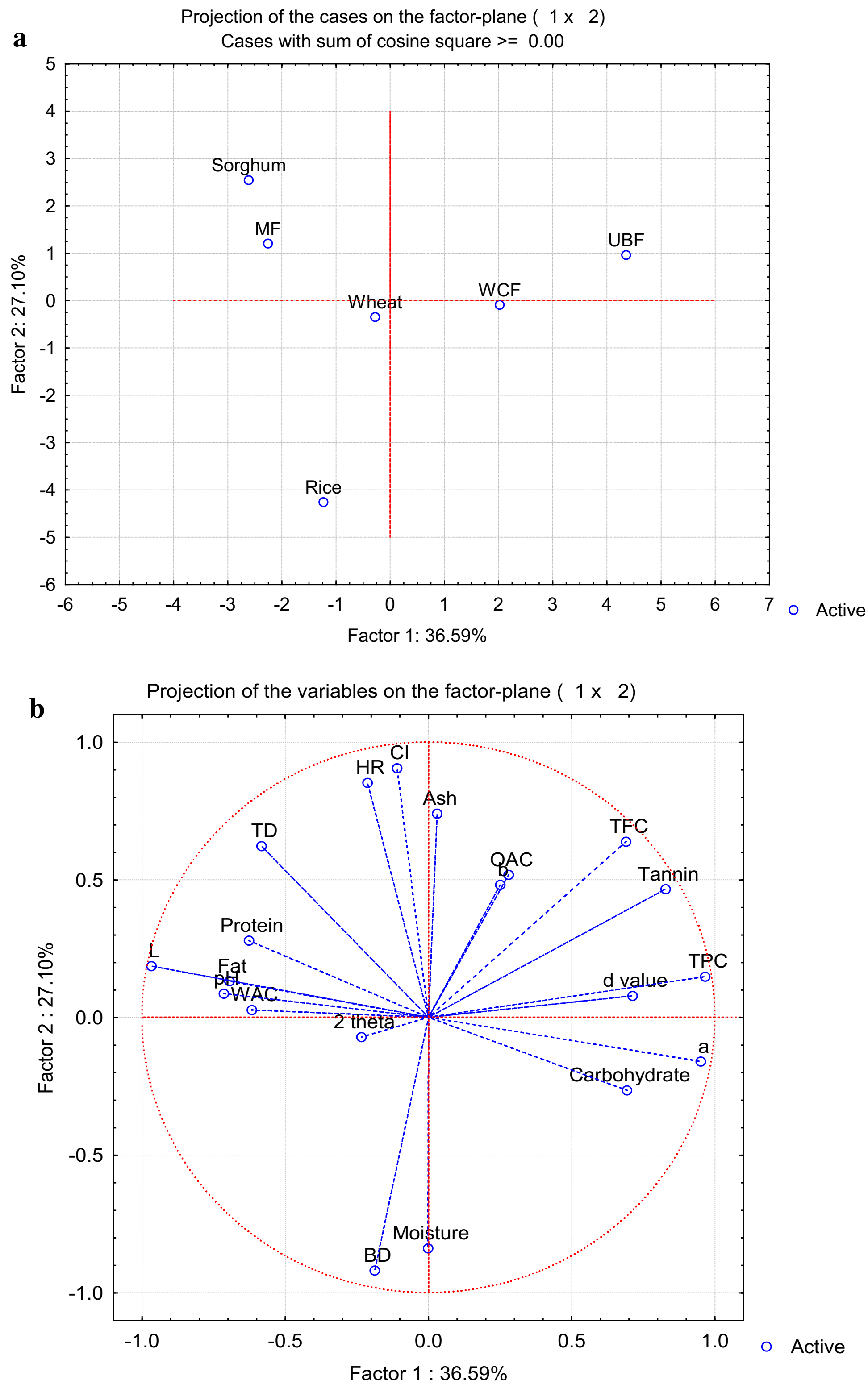


**Fig. 3** **a** Principal component analysis: score plot of first principal component (PC1) and second principal component (PC2) describing the overall variation among flours. **b** Principal component analysis: loading plot of PC1 and PC2 describing the variation among the properties of flours. *MF* moong flour, *WCF* water chestnut flour, *UBF* unripe banana flour, *BD* bulk density, *TD* tapped density, *HR* Hausner's ratio, *CI* Carr's index, *OAC* oil absorption capacity, *WAC* water absorption capacity, *TPC* total phenolic content, *TFC* total flavonoid content

## Conclusion

Present investigation shows that gluten-free flours vary to certain extent in the various characteristics studied. Gluten-free flours except sorghum have lower density, carr's index and hausner's ratio compared to wheat. Wheat and non wheat samples were characterised by high cohesiveness and poor flowability. The study of bioactive constituents showed their higher concentration in UBF and WCF compared to wheat. XRD study reveals their varying crystallinity patterns and d spacing values as compared to wheat. Lower densities of rice, MF, WCF and UBF make flours suitable for the formulation of high nutrient density weaning food. Gluten-free flours could be used as thickener in liquid/semi-liquids foods since the flours has the ability to absorb water and swell for improved consistency in food. This ability make them to be used as potential constituent in bakery products such as sandwich bread, sweet bread, butter cake, chiffon cake and instant fried noodles. Also, oil absorption capacity values make them advantageous as functional ingredient in foods such as whipped toppings, sausages, sponge cakes etc. PCA revealed the similarities between MF and WCF with respect to wheat. Altogether, this study concludes that wheat can be substituted by these flours in various applications.

**Acknowledgements** Authors thank University Grants Commission, Government ofIndia for financial support.

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