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Comparative study of nutritional and functional characteristics of pearl millet, buckwheat, amaranth and unripe banana flours for gluten-free bakery products

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Abstract

Suburbanization and changes in societal habits have led to a paradigm shift towards products that are nutritionally rich and therapeutic. However, the indispensable wheat flour in the bakery industry is also a trigger for gluten-related disorders. The present research focused on the characterization of nutritional and functional properties of gluten-free cereals-pearl millet, buckwheat, amaranth and unripe banana flours in comparison with wheat flour. The data were evaluated using analysis of variance and the difference between specific pair of means was compared using Duncan's multiple range test at a 95% confidence interval. Pearson correlation analysed the interaction amongst derived parameters. The results were significantly different ($p<0.05$) except for oil-absorbing capacity. The gluten-free flours reported higher protein (15.55% for amaranth), carbohydrates (82.69% for unripe banana) and energy content (384.72 kcal/g for pearl millet) compared to wheat flour whereas the moisture (6.26% for pearl millet) and fat content (0.98% for unripe banana) were lower. Amaranth had the highest water holding capacity and foam capacity (3.86 mL/g and 26.62% respectively) but the lowest foam stability (89.20%). Pearson correlation showed significant relations among functional properties and food components. Oil-absorbing capacity was found to be positively correlated to protein and negatively to carbohydrates and foam stability. The foam capacity was strongly correlated with different food components. The protein-rich and high energy gluten-free flours along with their functional properties can aid in the development of composite flour and fill the gap in the demand-supply of the therapeutic gluten-free diet.

Introduction

Wheat flour has been used in the bakery industry since time immemorial providing airy and light crumb structured products. However, its use worsens health conditions in people suffering from gluten-related disorders comprehending Celiac Disease (CD), Non-celiac Gluten Sensitivity (NCGS), Gluten Ataxia and Wheat Allergy. Furthermore, over-exploitation and over-consumption of wheat have led food researchers to look for other alternatives to combat malnutrition and food insecurity in many countries. Wheat flour is fortified to boost its micro-nutrient composition whereas recent studies (Bouasla and Wójtowicz, 2019; Bouasla and Wójtowicz, 2019; Khoozani *et al.,* 2020; Martínez-Castaño *et al.,* 2020) have shown the use of cereals (rice, maize, sorghum), pseudocereals (buckwheat, amaranth, quinoa), millets (pearl, foxtail, kodo) and fruits and vegetables by-products (chestnut, unripe banana, orange pomace) to provide a nutritionally rich alternative to wheat-based bakery products.

Pearl millet accounts for 50% of the global production; India is the largest producer (Venkata Rao *et al.,* 2018). It is a poor man's dependable source of energy and satisfies valuable dietary needs related to iron, calcium, zinc and good quality protein (Dias-Martins *et al.,* 2018). Additionally, with its antioxidant property, it can play a pivotal role in the nutraceutical sector (Nambiar *et al.,* 2011). *Bajra* helps in effective diabetic management since many studies (Kam *et al.,* 2016; Almaski *et al.,* 2019) have indicated decreased glycaemic response and improved heart health. An alternate pseudo-cereal, buckwheat, is an underutilized crop in Asia, Central and Eastern Europe. Of late, buckwheat has gained popularity owing to its nutritive

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value and role as a functional food in human diseases (Kumari and Chaudhary, 2020). Another pseudocereal to be rediscovered in recent times includes amaranth having higher protein than most cereal grains. Its protein profile is comparable to that of an egg (Mithila and Khanum, 2015). Unlike cereals, pseudocereals are a reservoir of lysine, zinc, calcium, iron, magnesium, essential vitamins, antioxidants, flavonoids, phytosterols and fibre (Pirzadah and Malik, 2020; Rodríguez *et al.,* 2020). Thus, they act as active health support aiding in cholesterol, blood pressure and ease in digestibility (Pavlik, 2012; Pirzadah *et al.,* 2017). Fruit and vegetables' flours present themselves as latent clear avenues for the formulation of gluten-free products. Green banana flour has little or no commercial value but is a cheap alternative. Moreover, it is an essential source of macro- and micro-nutrients such as potassium, zinc, calcium, vitamin C, and B6 (Suntharalingam and Ravindran, 1993; Falcomer *et al.,* 2019). The bioactive compounds and indigestible resistant starch confer additional health benefits in treating type-2 diabetes, preventing intestinal cancer and blood cholesterol reduction (Bodinham *et al.,* 2010; Ble-Castillo *et al.,* 2010; Dan *et al.,* 2015; Silva *et al.,* 2016). Unripe banana flour is a high-energy, calorie surplus source which can be a boon to developing nations. A collaborative approach can thus help to explore these underutilized crops and synergize their use with main cereals to augment the present food bag and provide food and nutritional security blanket. Moreover, the lack of gluten in these flours makes them the appropriate food for gluten-intolerance patients.

The variety of gluten-free flours and the minimal work on their characteristics demands the study of their nutritional and functional properties which will further broaden their applications as an alternative in the food industry. Proximate analysis data provide information regarding the macronutrients in food samples (Aziah and Komathi, 2009). Functional analyses determine the complex interaction among food components, structure and molecular conformity. It enables the determination of behaviour among new protein, carbohydrate, fat and fibre together with their interaction in the food matrix during preparation and effect on finished food products (Awuchi *et al.,* 2019; Naiker *et al.,* 2019). In the gluten-free system, the study of functional properties may predict whether or not a new protein system can stimulate or replace gluten.

The objective of the present study was therefore to evaluate the nutritional and functional characteristics of gluten-free flours encompassing pearl millet, buckwheat, amaranth and unripe banana flour in comparison with wheat flour and to analyse the interaction between derived parameters.

2. Materials and methods

Whole buckwheat, pearl millet, amaranth and wheat flour were purchased from CropConnect (Delhi, India) in a single lot. Unripe banana flour was obtained from Koko's Natural (Mumbai, India). Chemicals used in this study were of analytical grade from Sigma–Aldrich Co. (Steinheim, Germany).

2.1 Nutritional analyses

The different flours were analysed in triplicate for moisture, ash, crude protein, crude fat, crude fibre and carbohydrate contents following Association of Official Analytical Chemists (AOAC, 2005) protocols. The flours were dried at 105°C for 3 hrs using the oven drying method. Protein content was determined using the Kjeldahl method and factor 6.25 was used to calculate the nitrogen content. Fat content was determined using the Soxhlet extraction method. The ash was determined using the muffle furnace at 550°C for 2 hrs. For crude fibre, the flour sample was initially digested with acid, and then washed with distilled water. Furthermore, the sample was alkali digested and again washed with distilled water. The residue was dried in a hot air oven and fibre was calculated. Carbohydrate content was calculated by difference where carbohydrate = [100 - (moisture + ash + protein + fat + fibre)] (Ranganna, 1986). The results were expressed as % w/w. The Atwater factor was used to compute the energy content (each fat value were multiplied by 9 kcal/g while carbohydrate and protein values were multiplied by 4 kcal/g).

2.2 Functional analyses

2.2.1 Bulk density

The bulk density (BD) of the samples was determined using the method described by Oladele and Aina (2007). About 50 g flour sample was weighed in a 100 mL measuring cylinder. The sample was gently tapped continuously until a constant volume was obtained.

$$\text{Bulk Density, g/mL} = \frac{\text{weight of sample}}{\text{volume of sample after tapping}}$$

2.2.2 Water absorption capacity

Water absorption capacity (WAC) is defined as the maximum quantity of water taken up by the food in presence of an excess of water, with hydration time as less than an hour and an external force. It was measured according to Diniz and Martin (1997) methodology. About 0.5 g flour sample was suspended in 10 mL water in a centrifuge tube and agitated for 30 s. The dispersion

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was allowed to stand for 30 mins and then centrifuged at 3000 rpm for 25 mins. The supernatant was filtered using Whatman No.1 filter paper and the volume recuperate was measured.

$$\text{WAC, mL/g} = \frac{\text{Water absorbed}}{\text{Weight of sample}} \times 100$$

2.2.3 Oil absorption capacity

Oil absorption capacity (OAC) was conducted using the AOAC (2005) method. Approximately 1 g of flour sample was measured in a pre-weighed centrifuge tube and stirred with 10 mL refined groundnut oil using a vortex mixer. The sample was allowed to stand for 30 mins and then centrifuged at 3000 rpm for 20 mins. The supernatant was decanted and the volume was recorded.

$$\text{Oil Absorption Capcity, mL/g} = \frac{\text{Volume of supernatant oil}}{\text{Weight of sample}} \times 100$$

2.2.4 Foam stability and foam capacity

The foaming properties i.e. Foam capacity (FC) and stability (FS) were determined using the protocol discussed by Naiker *et al.* (2019). FC is defined as the amount of interfacial area that can be produced by whipping the protein in food. FS is the time required to lose either 50% of the volume or 50% of the liquid from the foam. The flour sample (2% w/v) was homogenised for 3-5 mins to form foam and the total volume was recorded after 30 s.

$$Foam\ Capacity, \% = \frac{Volume\ after\ homogenization - Volume\ before\ homogenization}{Volume\ before\ homogenization} \times 100$$

FS was calculated by leaving the sample undisturbed at ambient temperature for 60 mins.

$$Foam\ Stability, \% = \frac{Volume\ after\ 60\ mins}{Volume\ before\ homogenization} \times 100$$

2.3 Statistical analysis

The data collected from the nutritional and functional analyses of different flours were recorded as mean ± SD (standard deviation) and subjected to analysis of variance (ANOVA) and the difference between specific pairs of means was analysed using Duncan's multiple range test at 95% confidence level ($p < 0.05$) using IBM SPSS software (IBM Cooperation, New York, USA). The association between food constituents and functional characteristics of flour was determined using Pearson's correlation.

3. Results and discussion

In the present work, different gluten-free flours – pearl millet, buckwheat, amaranth and unripe banana flour were analysed for their nutritional and functional characteristics to explore alternatives of wheat flour for gluten-free bakery products (Table 1). The correlations among different food components and functional properties are depicted in Table 2.

3.1 Nutritional analyses

The moisture content ranged from 12.74% in wheat flour to 6.26% in pearl millet flour (Table 1). Moisture plays a major role in affecting storage stability and less than 14% is deemed favourable to prevent deterioration and enzyme activities (Ohizua *et al.*, 2017). Comparable results ranging from 11.16 to 13.29% for wheat flour were reported by other researchers (Aziah and Komathi, 2009; Baljeet *et al.*, 2010; Bashir *et al.*, 2017). Elsewhere, other groups of researchers (Ohizua *et al.*, 2017; Alonso-Miravalles and O'Mahony, 2018), studied the moisture content of various flours including quinoa, amaranth, buckwheat, sweet potato and pigeon pea. The results were akin to our study.

Table 1. Nutritional and Functional properties of different types of flours

	Pearl Millet	Buckwheat	Amaranth	Unripe Banana	Wheat
Nutritional Analysis (%)					
Moisture	6.26±0.26^{a}	8.65±0.11^{c}	7.51±0.17^{b}	8.49±0.17^{c}	12.74±0.17^{d}
Ash	1.70±0.17^{c}	1.47±0.07^{b}	2.86±0.07^{d}	1.05±0.04^{a}	0.96±0.06^{a}
Protein	10.73±0.23^{c}	14.21±0.11^{d}	15.55±0.30^{e}	5.45±0.29^{a}	10.21±0.14^{b}
Fat	4.93±0.07^{d}	2.30±0.41^{c}	5.27±0.19^{d}	0.98±0.03^{a}	1.41±0.16^{b}
Fibre	2.02±0.10^{c}	1.54±0.06^{b}	2.89±0.20^{d}	1.33±0.19^{ab}	1.07±0.16^{a}
Carbohydrate	74.37±0.41^{c}	71.82±0.67^{b}	65.91±0.47^{a}	82.69±0.69^{d}	73.60±0.37^{c}
Energy Value	384.72±1.18^{e}	364.85±1.64^{c}	373.25±1.35^{d}	361.37±1.70^{b}	347.99±0.35^{a}
Functional Analysis (%)					
Bulk Density	62.3±0.04^{a}	58.3±0.03^{a}	62.0±0.02^{a}	63.7±0.05^{a}	75±0.02^{b}
WAC	247±0.35^{b}	158±0.38^{a}	386±0.23^{c}	307±0.18^{bc}	155±0.40^{a}
OAC	110±0.11^{a}	153±0.36^{a}	163±0.44^{a}	104±0.06^{a}	138±0.35^{a}
FC	18.63±0.48^{c}	20.81±0.38^{d}	26.62±0.45^{e}	16.64±0.48^{b}	12.50±0.50^{a}
FS	95.42±0.48^{c}	93.53±0.44^{b}	89.20±0.65^{a}	96.82±0.35^{d}	96.19±0.25^{cd}

Values are presented as mean±SD of 3 replicates. Values with different superscripts within a row are significantly different ($p < 0.05$).

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Table 2. Coefficients of Pearson correlations between nutritional and functional properties of different flours

	Moisture	Ash	Protein	Fat	Fibre	CHO	Energy	BD	WAC	OAC	FC	FS
Moisture		-0.564*	-0.18	-0.681**	-0.652**	0.09	-0.943**	0.747**	-0.547*	0.116	-0.662**	0.345
Ash			0.728**	0.859**	0.962**	-0.775**	0.600*	-0.44	0.673**	0.352	0.916**	-0.934**
Protein				0.634*	0.636*	-0.960**	0.293	-0.315	0.047	0.588*	0.711**	-0.843**
Fat					0.885**	-0.687**	0.834**	-0.387	0.481	0.15	0.725**	-0.684**
Fibre						-0.688**	0.682**	-0.47	0.724**	0.233	0.900**	-0.831**
CHO							-0.245	0.145	-0.142	-0.553*	-0.662**	0.857**
Energy								-0.615*	0.436	-0.102	0.603*	0.359
BD									-0.294	0.045	-0.657**	0.376
WAC										0.029	0.624*	-0.506
OAC											0.354	-0.534*
FC												-0.899**

CHO: carbohydrate, BD: bulk density, WAC: water absorption capacity, OAC: oil absorption capacity, FC: foam capacity, FS: foam stability, Correlations of > 0.50 are in bold, * significant at 5%, ** significant at 1%

The highest ash content was found in amaranth (2.86%) followed by pearl millet (1.70%), buckwheat (1.47%), unripe banana (1.05%) and wheat flour (0.96%) (Table 1). Shevkani *et al.* (2014) and Alonso-Miravalles and O'Mahony (2018) in their study reported 3.2% and 2.4% ash content in amaranth respectively which is in concordance with our study. Alonso-Miravalles and O'Mahony (2018) also studied ash content in buckwheat (1.51%) which is nearly in tandem with the present finding. Except for unripe banana flour and wheat flour, the other flours were found to be significantly different from each other at a 5% level. One of the reasons for high ash content could be the use of whole flours as the outer husk is a rich source of minerals (Lopera-Cardona *et al.,* 2016).

The total protein content analysis exhibited a statistically significant difference ($p<0.05$) among different flours (Table 1). The protein content among different flour revealed amaranth (15.55%) and buckwheat (14.21%) are exceedingly protein enriched in comparison to wheat flour (10.21%). Unripe banana flour had the least protein content (5.45%). Chauhan *et al.* (2016) studied and compared the proximate composition between wheat and amaranth flour and found similar results for protein. Shevkani *et al.* (2014) in their study highlighted the physiochemical properties of full-fatted and defatted amaranth flours and found protein content to be in the range of 12.3 to 16.7% among its different varieties. Amaranth protein is rich in essential amino acids, especially lysine which the other cereal grains lack and is considered beneficial for both healthy and diseased people (Pavlik, 2012). The protein content in flours other than wheat justifies the need for composite/gluten-free formulations.

Further, the low protein content in unripe banana flour could be attributed to changes in physicochemical and functional characteristics of bananas at different ripening stages, the protein content increases with the 2nd and 3rd ripening stages (Campuzano *et al.*,2018).

The fat content varied from 0.98% to 5.27%, with unripe bananas having the least and amaranth the highest (Table 1). One of the reasons for higher fat content could be the inclusion of the outer husk of grains. Other than moisture content, fat content below 5% aids in shelf-life stability. Moreno *et al.* (2014) reported the average fat value for pearl millet, buckwheat and amaranth as 4.86%, 2.10% and 5.70% respectively. In another study (Bashir *et al.*, 2017), the result for wheat flour fat was in parallel to our results at 1.37%.

The crude fibre content for gluten-free flours was higher than wheat flour (1.07%), amaranth having the highest (2.89%). The crude fibre depicted a correlation with all the other nutritional food components and had a significant positive correlation with foam capacity as well.

The carbohydrates ranged from 65.91% for amaranth flour to 82.69% for unripe banana flour (Table 1). One of the major reasons for the high carbohydrate content in unripe banana flour can be accredited to the high level of dietary fibre and resistant starch in it (Borges *et al.,* 2009; Hettiaratchi *et al.,* 2011; Zandonadi *et al.*, 2012; Falcomer *et al.,* 2019). A few studies have reported starch content of unripe banana flour in the range of 72-83%. A strong negative correlation is observed between carbohydrate and protein ($r = -0.959$, $p<0.01$) and with fat ($r = -0.639$, $p<0.05$) (Alviola and Monterde, 2018; Marta *et al.,* 2019).

The energy values of flours differed significantly ($p<0.05$) varying from 347.99 kcal/g of wheat flour to 384.72 kcal/g of unripe banana (Table 1). A positive Pearson's correlation is observed between energy value and fat and fibre ($r_{(fat)} = 0.834$, $r_{(fibre)} = 0.682$ $p<0.01$). The high-energy gluten-free flours can be productive for product formulation since energy intake is scarce in celiac patients before and during the GF diet (Vici *et al.,* 2016).

On the whole, gluten-free flours are naturally rich in protein and carbohydrates which lack in the gluten-free diet. For designing a product, nutritional analyses play a crucial role as it summarizes the nutritional quality of food components. For example, formulating a protein-energy-rich product can utilize a composite flour of amaranth and unripe banana. Different food components affect various functional properties as well.

3.2 Functional analyses

The individual attributes of the finished product are influenced by the functional properties of the raw ingredients, as well it plays a major role to ascertain their end-use (Awuchi *et al.,* 2019). As depicted in Table 1, a significant difference ($p<0.05$) existed for all functional properties except that of OAC of flours. Different nutritional parameters influence the functional properties; the association of which is delineated in Pearson correlation (Table 2).

The results of bulk density (BD) indicate no significant differences amongst gluten-free flours whereas the BD of wheat flour differed significantly. The wheat flour showed the highest BD (0.75 g/mL). A positive and strong correlation (r = 0.747, p<0.b 01) is calculated between BD and moisture content which are in accordance with Chandra and Samsher (2013). This functional property affects the porosity of the food product and its application to the type of food formulation as well as helps to choose the type of packaging material (Awuchi *et al.,* 2019). In a study, Baljeet *et al.* (2010) studied the functional properties of buckwheat flour and wheat flour to be incorporated into cookies. The results for BD of buckwheat flour were similar to the present study but higher BD was obtained for buckwheat flour. However, increased BD is not favourable because higher density deters the ability to compress flour, thus, increasing the price of packaging.

WAC plays a pivotal role in enhancing conformity in food. The WACs ranged from 1.55 mL/g in wheat flour to 3.6 mL/g in amaranth. Amaranth and unripe bananas show higher WAC due to the presence of comparatively higher starch content as discussed in the literature (Pavlik, 2012; Alviola and Monterde, 2018). Protein and starch enhance WAC as their hydrophilic parts have a higher affinity for water molecules (Awuchi *et al*., 2019). Higher WAC allows the addition of more water to boost dough handling as is required in most gluten-free formulations which form batter rather than dough. Hence, the higher WAC of gluten-free flours than wheat flour makes them good potential in baked products (Adebowale *et al.,* 2005). The results are in line with previous studies (Rai *et al.,* 2014; Jan *et al.,* 2015; Chauhan *et al.,* 2016; Ohizua *et al.,* 2017) where WAC were 1.27, 2.23, 2.99 and 1.80 mL/g for buckwheat, pearl millet, unripe banana and wheat flours respectively.

The results for OAC properties for different flours ranged from 1.04 mL/g (unripe banana flour) to 1.63 mL/g (amaranth flour) with no significant difference ($p<0.05$). Positive Pearson's correlation existed between protein content and OAC ($r = 0.588$, $p<0.05$). This can be explained by the twin hydrophilic and hydrophobic nature of the protein. The non-polar amino acid chain allows binding with the hydrocarbon chain of fat (Naiker *et al.,* 2019) and thus, the highest OAC of amaranth can be attributed to its high protein content (15.55%). Similar trends of increasing OAC with increasing protein content were described by Siroha *et al.* (2016) in their study on different varieties of pearl millet. Likewise; Jan *et al.* (2015) in their study replaced wheat flour with buckwheat flour and reported similar observations. Moreover, Chandra and Samsher (2013) addressed proximate and functional properties of different flours and obtained alike OAC values. Flours with high OAC are useful in gluten-free systems for enhancing flavour and improving mouth feel.

The foaming properties include FC and FS. Good foaming properties of flour are advantageous for use in the baked product especially gluten-free since they influence gas holding capability and gas cell expansion during kneading and proofing (Ziobro *et al.,* 2013; Horstmann *et al.,* 2017). The highest FC was observed in amaranth (26.62%) followed by buckwheat (20.81%), pearl millet (18.63%), and unripe banana (16.64%) and the least wheat flour (12.50%). The FC of amaranth can be ascribed to its high protein content. At the air-water interface, the proteins unfold/refold enhancing their capacity to enfold more air bubbles. The high positive correlation ($r = 0.711$, $r<0.01$) between protein content and FC also establishes the same. FC shows positive correlation with ash ($r = 0.916$, $p<0.01$) and fat ($r = 0.725$, $p<0.01$) but negative correlation with moisture ($r = -0.662$, $p<0.01$), carbohydrate ($r = -0.662$, $p<0.05$) and BD ($r = -0.657$, $p<0.01$). Our results are similar to that of Chandra and Samsher (2013), Lopera-Cardona *et al.* (2016), Ohizua *et al.* (2017) and Naiker *et al.* (2019) who concluded that high protein favours FC.

The FS of different flours were significant at a 5% level. The least FS was induced by amaranth (89.20%) and the most by unripe banana (96.82%). A high negative correlation ($r = -0.899$, $p<0.01$) was found between FC-FS and FS-Protein ($r = -0.843$, $p<0.01$)

which is in line with the finding of Awolu (2017). Flours that foam large air bubbles have thin and weak protein film resulting in closely-packed unsteady foams. This result is in accordance with Naiker *et al.* (2019). Correlations between carbohydrate-FS were found to be highly positive (r = 0.821, p<0.01) which is parallel with the reports of Damodaran (2017). Carbohydrates weaken the foam formation but help in foam stability by lessening the rate of discharge of lamella fluid due to bulk phase viscosity surge (Naiker *et al.,* 2019). These results suggest the use of hydrocolloids which will mimic gluten and improve the gas retention properties of a gluten-free system.

As seen in the results of nutritional and functional analyses varied correlations exist amongst different properties. Correlations among different nutritional and functional properties (Figure 1) shows that moisture is inversely linked to ash (r = -0.564, p<0.05), fat (r = -0.681, p<0.01), energy (r = -0.674, p<0.01) and WAC (r = -0.662, p<0.01). Ash was directly proportional to protein (r = 0.728, p<0.01), fat (r = 0.859, p<0.01), WAC (r = 0.673, p<0.01) and FC (r = 0.916, p<0.01). This means that flour with high ash will have high protein, fat and better water-holding ability. These results are similar to the report where physiochemical and functional properties of chemically pre-treated Ndou sweet potato flour were assessed. (Ngoma *et al.,* 2019).

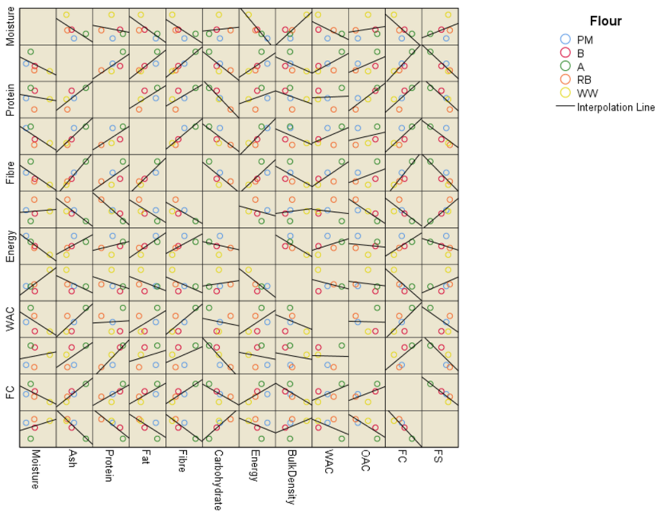

Figure 1. Scatter Plot between nutritional composition and functional properties of pearl millet, buckwheat, amaranth, raw banana and wheat flours

4. Conclusion

The present study characterized the nutritional and functional properties of gluten-free cereals - pearl millet, buckwheat, amaranth and unripe banana flours in comparison with wheat flour. On the whole, the use of these gluten-free flours in combination points towards economic and healthy alternatives for wheat flour in the bakery industry. These can aid in the development of composite flour to fill the gap between the demand and supply of wheat alternate therapeutic baked products to suit consumers' acceptance. However, further study on vitamins, minerals and bioactive compounds of gluten-free flours is suggested to explore their micro-nutritional aspects in detail.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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