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Nutrient and phytonutrient quality of nutricereals incorporated flour mix suitable for diabetics

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Abstract

Diabetes is a health problem whose burden is evident in developing countries, and over the decades, it has been drastically increasing at the global level. It became one of the causes of death. Diet is one of the interventions to control diabetes. Consumption of millets or whole grains with pulses is associated with lowering non-communicable diseases, as they contain a large amount of dietary fiber, minerals, phenolics, and antioxidants. The current study was aimed to develop a product from millets suitable for consumption by people with diabetes. Selected millets, *viz.*, foxtail millet, proso millet, kodo millet and barnyard millet were procured from the local market. Wheat flour (*Triticum aestivum* L.), green-gram dhal (*Phaseolus aureus* Roxb), oats, soybean (*Glycine max* Merr), barley, fenugreek seeds, and gums were procured from the local market. Flour mixes were prepared by mixing dehulled, milled millet flours with other grain flours in different proportions, keeping 100% whole wheat flour as control. All the flour samples were tested for nutrients and phytonutrients using AOAC methods. The moisture content, total carbohydrate, and energy content were decreased; protein, crude fiber, ash, and lipid contents were increased significantly ($p<0.01$) with the incorporation of millet flour blend in minor millet flour mix (MMFM) compared to control flour. Antioxidant and phytonutrients, *viz.*, total phenols, and phytic acid content were high in the MMFM.

1. Introduction

Diabetes is a global public health problem whose burden is evident in developing countries such as India (Kaveeshwar and Cornwall, 2014). According to the Indian Diabetes Federation (2019), 463 million people had diabetes in the world. The global prevalence of diabetes is estimated to be 578 million by 2030, and India had an 8.3% prevalence with the second rank in the world. One of the major strategies in controlling diabetes is maintaining effective glucose levels. Fasting plasma glucose ≥ 126 mg/dl after two hours post-load glucose ≥ 200 mg/dl, HbA1c ≥ 6.5% (ADA, 2018). Regular consumption of plant-based foods containing bioactive compounds like protein, fiber, and phytochemicals are associated with fewer digestive disorders, reduced colon cancer rate, better blood-sugar control, and lower blood cholesterol levels.

The management of Type II diabetes mellitus needs diet modification which includes a diet high in complex carbohydrates, protein, fiber, and low in fat, which does not cause a rapid rise in blood glucose levels. The slower the rate of carbohydrate absorption, the lower is the rise in blood glucose level and the glycaemic index value. Significant alterations in the glycemic index and dietary fiber content of meals were known to include small but significant glucose profile changes (Karuppaswamy, 2013). Millets are small seeded grains with a rich dietary fiber source that provides a wide range of nutrients and phytochemicals, including vitamin E, magnesium, and folate that optimize health. Millets contain a higher proportion of unavailable carbohydrate and the release of sugar from millets. They contain water-soluble gum and β-glucan which help improve glucose metabolism. Millets contribute to antioxidant activity through phytates, polyphenols, and tannins present in them, having an essential role in ageing and metabolic diseases (Bravo, 1998). Therefore, millets are suitable for a diabetic diet, but the characteristic flavour and difficulty in the processing are the limitations for its incorporation into the diet (Vijayalakshmi and Radha., 2006). Millets are called diabetic-friendly grains due to high macronutrients and dietary fiber content, low digestion, and low sugar release and millets have a low glycemic index compared to other cereals. The legumes and pulses have been known as a poor man's meat. They supply protein, complex carbohydrates, fiber, and essential vitamins and minerals to the diet, which are low in fat and sodium and contain no cholesterol (Priyanka *et al*., 2018).

Millets are traditionally used in the form of unleavened bread (roti), dumplings, porridge, and desserts. Most commonly followed conventional processing methodologies for millets are milling, including decortications and size gradation, popping, malting, fermentation, and cold extrusion (Kotagi *et al*., 2013). Composite

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flour technology refers to the process of mixing various cereal-based flour with pulse and legume flours to produce high-quality food products economically. Formulation of composite flour is vital for the development of value-added products with optimal functionality. They have not only better nutritional quality but also the necessary attributes for consumer acceptance. The nutritional value for cereal flours that are poor in lysine but rich in sulfur-containing amino acids is improved by adding legume flours (Kadam *et al*., 2012). A multigrain flour helps in a high intake of fiber and health-enhancing components (Ragaee and Abddel Aal, 2006).

Hence, the present study was taken up to develop value-added minor millet flour mix by using the locally available cereals, millets, pulses for multipurpose usage and fit into the people's general lifestyle.

2. Materials and Methods

2.1 Raw materials procurement and sample preparation

Whole foxtail millet, proso millet, kodo millet, and barnyard millet were procured from the local market. Millet grains were dehulled in an abrasive dehuller (Gurunanak Engineering Co, Hyderabad) to achieve 17% removal of the bran and the dehulled grains collected through bottom opening along with hull. Then to obtain dehulled grain, bran was separated by winnowing. Wheat flour (*T. aestivum*), green-gram dhal (*P. aureus*), oats, soybean (*G. max*), barley, fenugreek and gums seeds were procured from the local market. An electronic weighing balance (Model AB204) was used to weigh the chemicals and samples for the study. Hot air oven (Deccan Coporation ISO 9001:200 certified company) was used to determine samples' moisture content. Gallic acid, Folin-Ciocalteu's reagent, phytic acid, and enzymes for digestion of starch and dietary fiber estimation, pepsin, pancreatin, Termamyl (A 3403), and amyloglucosidase were from M/s Sigma Chemical Co. (St. Louis, MO, USA). All the chemicals used for the study were laboratory reagent (LR) and analytical reagent (AR) grades.

Table 1: Ingredients proportions for preparation MMFM

Ingredients	MMFM (%)
Foxtail millet	12.5
Proso millet	12.5
Kodo millet	12.5
Barnyard millet	12.5
Wheat	25
Green gram	6
Barley	6
Oats	6
Soya	5
Fenugreek	1
Gums	1

2.2 Preparation of flour *mix*

All dehulled millets and other grains were dried in tray dryer (Sowbagya Industries, Kolakalur, Tenali)) for 4 h at low temperature (40°C) and each grain was milled into flour in a pulveriser (Able manufacturers, Hyderabad). All flours were mixed in different proportion as mentioned in Table 1, then mixed flour mix was sieved through 40 size mesh toobtain uniform sized particles. The control mix was prepared using 100% wheat flour and MMFM (minor millet flour mix) was prepared as per the proportions shown in Table 1. The developed mixes were packed in a polythene (PE) bag and stored at 30°C until further analysis.

2.3 Proximate analysis

Proximate content of flour mixes were analyzed using standard protocols.

2.3.1 Moisture

Standard protocol (IS:1155-1968 reaffirmed 2010) was used for moisture estimation. Briefly, 5 g of the sample was dried at 105 ± 3°C for 2 h in a hot air oven. After 2 h, sample was cooled in a desiccator and weighed. This was repeated at 30 min intervals till constant weight was achieved. The moisture content of the sample was expressed in g/100 g of sample.

2.3.2 Protein

Crude protein was estimated using the microKjeldahl method (AOAC 954.01-2010) using Pelican Kelplus equipment, calculated by multiplying with a factor N×6.25 expressed as g/100 g.

2.3.3 Fat

Soxhlet method (AOAC 922.06 - 2007) was employed to estimate fat content and expressed as g/100 g.

2.3.4 Crude fiber

Moisture and fat-free samples were utilized for estimating crude fiber. Samples were treated with acid followed by an alkali bath as described in standard AOAC 962.09 - 2007 protocol. The residue obtained after final filtration was weighed, incinerated, cooled, and weighed again. Crude fiber content was expressed as g/100 g of the sample.

2.3.5 Ash

Total ash was estimated using a standard protocol (IS:1155-1968 reaffirmed 2010), where 5 g of dried sample was weighed in a crucible, ignited till it was charred completely. The charred sample was then transferred to the crucible and kept in the muffle furnace for about 4 h at 600°C till white or greyish color ash was obtained. It was then cooled in a desiccator and weighed after reaching room temperature.

2.3.6 Total carbohydrate (g/ 100 g)

100 – [Protein (g) + Fat (g) + Crude fibre (g) + Ash (g) + Moisture (%)]

2.3.7 Computation of energy

Energy was calculated for all the samples with the following formula:

Energy (Kcal/100 g) = [Protein (g) x 4] + [Carbohydrate (g) × 4] + [Fat (g) × 9]

2.4 Estimation of mineral content

Moisture-free flour samples were wet digested in a microwave digester using nitric acid. Further iron, calcium, zinc content was determined as described in standard protocol (AOAC 975.03) using atomic absorption spectrophotometry and sodium, and potassium were estimated using flame photometry (AOAC, 956.01).

2.5 Estimation of total dietary fiber

Total dietary fiber was determined using a standard protocol (Asp and Johansson, 1981), based on enzymatic and gravimetric methods. The samples (moisture and fat-free) were weighed accurately, subject to gelatinization with heat-stable a-amylase, and then digested with protease followed by amyloglucosidase for removing starch and protein content in the samples. Further, the samples were treated with ethanol for the precipitation of soluble dietary fiber. The residue was then filtered, washed with ethanol and acetone, followed by drying. Half of the samples were then analyzed for protein and another half for ash. Total dietary fiber was calculated as the weight of residue minus the weight of protein and ash.

2.6 Sample extraction for antioxdiants and phytonutrient analysis

Phytonutrients (Total phenols, flavonoids and phytic acid) and antioxidant activity (DPPH) was estimated for both samples.

Flour samples were accurately weighed (0.5 g) in a conical flask to which 15 ml of 80 % acidified methanol (pH 2.0) was added. The mixture was extracted by continuous shaking (155 rpm) at room temperature for 30 min, and the supernatant was collected. The residue was re-extracted twice, and all three collected supernatants were centrifuged at 6000 rpm for 15 min and filtered using Whatman No. 1 filter paper. The volume of the extract was noted and made to 50 ml using a solvent. The extracts were transferred to centrifuge tubes and stored at –20°C till further use. This extract was used to determine total phenol (TP), flavonoids and 1,1-diphenyl-2-picrylhydrazyl (DPPH).

2.7 Estimation of total phenoliccontent (TPC)

Total phenolic content of samples was determined using Folin–Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard phenolic compound. Briefly, 1 ml of approximately diluted samples and a standard solution of gallic acid were added to a 25 ml volumetric flask containing 9 ml of distilled water. A reagent blank using distilled water was prepared. 1 ml of Folic-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of a 7% Na_2CO_3 solution was added with mixing and then allowed to stand for 2 h. The absorbance was measured at 760 nm using Shimadzu UV spectrophotometer (model: UV-1800, Japan). The gallic acid standard curve (5 µg-35 µg) was prepared. TP content was determined from standard curve ($y = 0.0113x + 0.0148$; $R^2 = 0.992$) and expressed as mg gallic acid equivalent (GAE) mg/100 g of the sample. The concentration of total phenolic compounds in the samples was determined as a microgram of gallic acid equivalent using an equation obtained from a standard gallic acid graph.

Calculation: Std .conc/Std O.D /Aliquot taken * volume made up /sample taken *100/1000* dilution factor.

2.8 Estimation of total flavonoids content (TFC)

Flavoniod content of samples was estimated using Zhishen *et al.* (1999) method.

2.9 Estimation of DPPH radical scavenging activity (DSRA)

The flour mixes' free radical scavenging capacity was determined using 1,1-diphenyl-2- picryl- hydrazil (DPPH) (Dorman *et al.*, 2004). Two milliliters of a methanol solution of DPPH radical in the concentration of 0.05 mg/ml and 1 ml of extract were placed in cuvettes and allowed to stand at room temperature for 30 min after shaking. Then, the absorbance was measured at 517 nm against methanol as blank in a spectrophotometer. The DPPH free radical concentration was calculated using the following equation:

Calculation: percent inhibition = [(AC-AE)/ AC] *100

(where , AC = absorbance of control ; AE = Absorbance of extract)

2.10 Estimation of phytic acid

Phytic acid content of samples was estimated using Wheeler and Ferrel (1971) procedure.

2.11 Estimation of *In vitro* protein digestibility

IVPD of samples was estimated according to the procedure of Singh and Jambunathan (1981).

2.12 Estimation of resistant starch

Resistant starch estimated using Goni *et al.*(1996).

2.13 Statistical analysis

All experiments were performed three times. All data presented as means ± standard deviation of the mean. As for multiple group comparisons, the significance of the differences among the treatment groups and their respective control groups were analyzed using WINDOW STAT 9.1 software. Statistical significance was assessed by one-way analysis of variance (ANOVA). Differences between means were considered statistically significant at 5% level ($p < 0.05$).

3. Results

3.1 Proximate composition of the flour mixes

Grain quality, processing, the combination of grains, and the moisture content may affect the developed flour mix's nutritional composition. The proximate composition estimated, for each nutrient is summarized and presented in Table 1**.** All the values are presented on a dry weight basis.

3.1.1 Moisture

There was a significant difference ($p<0.01$) in moisture between the control flour 10.44 g/100 g and minor millet flour mix (MMFM) 9.47 g/100 g (Table 1). The present results were close to millet flours' moisture content (10.01% to 12.17%) reported by Singh *et al*. (2005). Chaudhary and Jood (2013) reported a higher moisture content in wheat flour.

3.1.2 Protein

Significantly ($p<0.05$) higher protein content was observed in MMFM (11.42 g/100 g) than that of control (7.39 g/100 g). The addition of different millets and pulses lead to a 35.2% increase in protein. The present results were in agreement with (Poongodi and Mohankumar, 2009) where, formulated millet based composite flour mix contained maximum protein. This increase in protein content in flour could be attributed to the significantly higher protein content of individual flour components, *viz*., soya flour that was incorporated in flour formulation. A high protein diet is good for the health of diabetics because it supplies essential amino acids needed for tissue repair. Protein does not raise blood glucose during absorption as do carbohydrates, and it does not supply as many calories as fats. In type 2 diabetics, protein and carbohydrate consumption lowers the blood glucose concentration sulfur-containing amino acid stimulation of insulin secretion and promotes satiety (Anitha and Philip, 2001; Srilakshmi, 2001).

3.1.3 Fat

Similar to protein, fat content was also significantly higher in millet flour mix (4.44 g/100 g) than that of control (1.5 g/100 g) (Table 2). Lakshmi *et al*. (2015) reported that compared to control bread (1.84 %), experimental composite flour bread (V-I, V-II and V-III were 3.37, 4.75 and 5.64%, respectively) had higher fat contents. It was observed from the results that the increase in fat content for the millet flour mix can be attributed to minor millets, which have higher fat content compared to wheat (Ahmed *et al*., 2013). As the increase in fat is not due to the external addition, there would not be any deleterious effects on health. This feature also highlights the possibility of using high-fat seed flours like millet as sources of natural fat replacers.

Fat delays gastric emptying delayed gastric emptying has the effect of decreased glycemic response to a meal. After a fat meal, gastric inhibitory polypeptide level increases; this potentiates glucose-induced insulin secretion initially, hence after 4hour fat may reduce insulin secretion, possibly due to the presence of chylomicrons or increased free fatty acids (Lin *et al*., 1996).

Table 2: Proximate composition of control flour and MMFM (%/100 g)

Samples	Moisture	Ash	Protein	Fat	Crude fiber	CHO(g)	Energy(Kcal)
Control flour	10.44	1.556	7.39	1.55	1.30	77.05	354.1
MMFM	9.472	2.263	11.425	4.488	5.23	67.07	270.72
S.E	0.04073	0.01504	0.03723	0.0782	0.027	0.05065	1.47984

S.E, Standard error; MMFM, minor millet four mix.

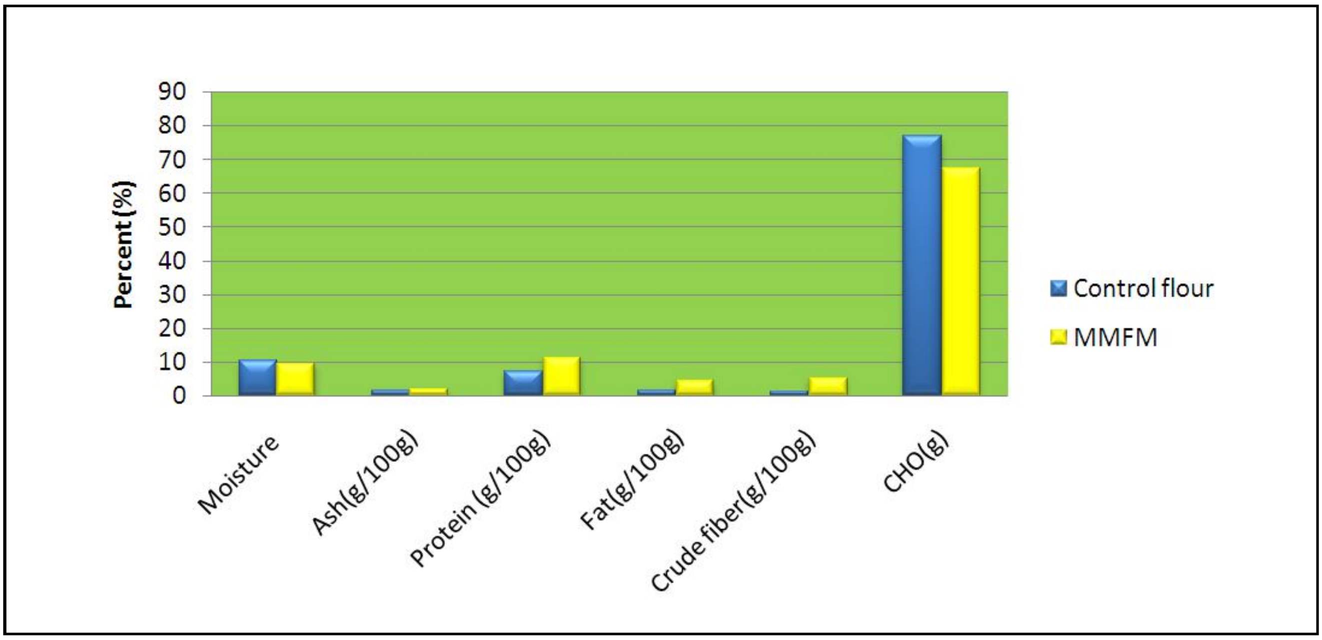

Figure 1: Proximate composition of flour mixes.

3.1.4 Crude fiber

Similar to protein and fat, the crude fiber content was also significantly (p<0.05) higher (75.1%) in MMFM (5.23 g /100 g) than in control flour mix with (1.3 g/100). Similar results reported by Laxshmi *et al*. (2015) fiber content in composite flour bread (1.9 %) and in three millet included bread variations as 2.1, 2.3 and 2.4% for V-I, VII and V-III, respectively. Thus, it can be attributed to fibre-rich millets grains. The fiber content increases significantly (p<0.05) due to the modification of the structure of cell wall polysaccharides of the seeds, possibly affecting the intactness of tissue histology and disrupting the protein carbohydrate interaction. This involves extensive cell wall biosynthesis and, therefore, new dietary fiber production (Martin-Cabrejas *et al*., 2003). The nutritional characteristics of composite whole wheat flour were gradually increased for dietary fiber, ash, protein, and fat contents. A blend of chickpea split without husk, barley, soybean, and fenugreek seeds was added from 0 to 40% (Indrani *et al*., 2011).

3.1.5 Carbohydrate

The control flour (77.0 g) had high carbohydrate, and MMFM (67.0 g) had lower carbohydrate, other pulse, legumes incorporation significantly reduced the carbohydrate content of experimental flour by 12.5% from control (Table 2). Similar results were reported by Deepa (2009) where, wheat flour (control flour) contained 70.37 per cent carbohydrate while 30 % incorporated millet based composite flour had 54.26 per cent of carbohydrate. Malathi *et al.* (2014) reported that 50% kodo millet incorporated *chapathi* had 54.12 of carbohydrate which is lower than the control chapathi, the difference in carbohydrate contents was attributed to the incorporation of low carbohydrate cereals in flour mix. In the present study, the decrease in the composite flour's carbohydrate content was due to the dilution effect of pulses, legumes, oats, and barley flours, which are low in carbohydrates. Complex carbohydrates are advised for diabetics; high fibre foods are encouraged to reduce the rate of absorption of glucose from the gut (Jenkins *et al.*, 1978).

3.1.6 Energy

The control flour (354.1) had high energy, and the MMFM had lower energy (270.7 Kcal) (Table.1); millet, pulse, legumes incorporation significantly (p<0.05) reduced the energy content of experimental flour with a percentage change of 23.6% from the control. Dietary management of energy (calories) intake is vital in diabetes (Deepa, 2009).

3.2 Minerals

The results presented in Table 3, exhibits a significant difference in minerals of control and millet flour mix.

Table 3: Mineral content of MMFM and control flour

Minerals	Control flour	MMFM
Ca(mg/100 g)	49.1 ± 0.24	52.9 ± 0.04*
Zinc(mg)	29.8 ± 0.38	33.3 ± 0.36*
Iron (mg/100 g)	4.67 ± 0.02	5.56 ± 0.08*
Potassium (mg/100 g)	34.7 ± 0.42	44.2 ± 1.58*
Sodium (mg/100 g)	0.02 ± .008	0.158 ± 0022*

Note: * = significantly different at 5 %

3.2.1 Calcium

The incorporation of millets and other grains in the wheat flour (p<0.01) affected the mix's calcium content. It was increased by 7.1% (from 49.16 to 52.95 mg/100 g of control and experimental, respectively). The results were in agreement with Karuppaswamy *et al.* (2013). Compared to the control bread (20.61mg), minor millets added formulations T1V1(kodo millet bread) (21.54 mg) and T2V2 (little millet bread) (22.21mg) had higher calcium content. The increasing trend in composite breads calcium content was possibly due to the higher calcium content of minor millets.

Kulakarni *et al.* (1991) reported that a multigrain mix contained higher calcium than little millet-based counterpart irrespective of green leafy vegetables used. Multigrain or little millet mix with chakramuni leaves had a significantly higher amount of calcium (61.46 mg and 60.12 mg/100 g, respectively), compared to amaranthus incorporated mixes (58.78 mg and 54.78 mg/100 g, respectively).

3.2.2 Zinc

It was observed that the zinc content was 33.31 mg and 30 mg/100 g) in MMFM and control flour, respectively. The results showed that incorporating millet significantly affected the millet flour mix's zinc content with a 9.24 per cent rise from control flour. Minerals are essential for body growth and development, functioning, immune functioning. Zinc plays an important role in the synthesis, storage, and secretion of insulin and conformational integrity of insulin in hexameric form. The decreased zinc affects the ability of islet cells to produce and secret insulin, particularly in type 2 diabetics (Chausmer, 1998).

3.2.3 Iron

From the results, it was observed that the incorporation of millet significantly influenced the content of different minerals in the minor millet based flour. MMFM had a significantly higher iron content than control flour by 46% as shown in Figure 2.

3.2.4 Sodium

The sodium content varied between flours and MMFM had higher (0.158 mg/100 g) than in control flour (0.02 mg/100 g) (Table.3). The variation in sodium content between samples was found statistically significant (p<0.05).

3.2.5 Potassium

The incorporation of millets and other grains in the wheat flour (p<0.01) affected the mix's potassium content. It was increased by 21.4% (from 34.76 to 44.29 mg/100 g of control and experimental, respectively). The variation between the samples was found statistically significant (p<0.05). According to Preuss (2001), a high intake of potassium can protect against increased blood pressure and other cardiovascular risks. Results indicated the addition of millets increased the potassium content of flour.

3.3 Resistant starch

Resistant starch (RS) was significantly high (p<0.05) in MMFM (32.4 g) than in control flour (27.52 g /100 g) (Table.4). RS is not digested and absorbed by humans as energy. It has a positive role against diabetes. Johnston *et al.* (2010) has discovered that the consumption of resistant starch improves insulin sensitivity. It does not aûect body weight, fat storage in muscle, liver, or visceral depots signiûcantly.

3.4 Dietary fiber

The MMFM had higher dietary fibre 21.158 g/100 g, and control flour had lower DF with 13.84/ 100g. Similar results reported by Hameeda *et al.* (2012) dietary fiber varied from 12.4 g -16.5 g in multigrian mixes.

3.5 *In vitro* protein digestibility (IVPD)

As shown in Table 4 IVPD content in control and MMFM was 55.0% and 62%, respectively. Results indicated that the addition of pulses, legumes could increase the protein digestibility of flours. Similar results were reported by Sujatha *et al.* (2015) were, the dehulled foxtail millet(56.0) had the highest IVPD followed by sorghum (55.8) and pearl millet (52.17). Similar results were also reported by Laminu *et al*. (2011) in pearl millet based weaning blends. However, the protein concentrates from yellow millet (foxtail millet), white millet (foxtail millet), soy beans were reported as 78%, 81, 84%, respectively Tabita *et al.* (2009).

Table 4: Resistant starch, IVPD, dietary fiber and phytonutrient content of flours

Samples	RS (%)	Dietary fiber(%)	IVPD (%)	Total phenols (GAE mg/100 g)	Antioxidants (mg/100 g)	Flavonoids (REmg/100 g)	Phytic acid (mg/100 g)
Control flour	27.52	13.85	55.43	106.9	85.70	152.0	206.4
MMFM	32.31*	21.16*	62.86*	240.2*	93.33*	164.0 *	278.2*
S.E	0.32526	0.11099	0.35061	0.04583	0.43462	0.63853	1.32626

RS: resistance starch; IVPD, *In vitro* protein digestibility; S.E, Standard error; GAE, Galic acid equivalent; RE, rutinequivalent; *, significantly different at 5% level(p<0.01).

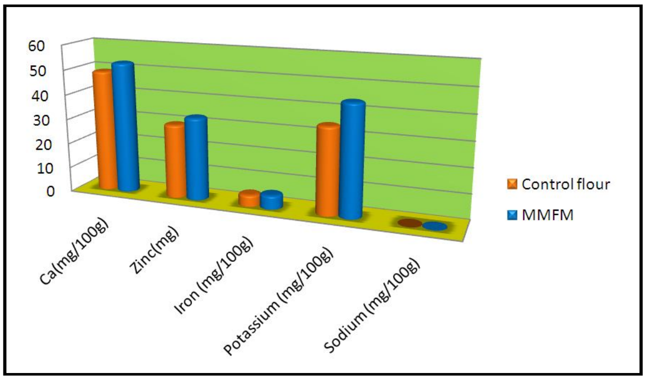

Figure 2: Mineral content of flour mixes.

3.6 Total phenolic content and flavonoids

Table 4 shows the total phenolic content of flour mixes. The total phenolic (240.2 GAE/100 g) and flavonoid (162 RE mg/100 g) content of the MMFM was found to be higher than that of the control mix (106. 9 GAE and 154 RE/100 g total phenol and flavonoids, respectively) (Table 4). Previously, Palanisamy*et al.* (2012) reported kodo millet as a rich source of phenolics in comparison to other millets. The outer layer of grain is considered the richest part in polyphenolic compounds (Awika *et al*., 2005; Liyana-Pathirana and Shahidi, 2007; Madhujith *et al*., 2006; Zielinski and Kozlowska, 2000). The main polyphenols in cereals are phenolic acids and tannins (Chandrasekara and Shahidi, 2010; Devi *et al*., 2011).

3.7 Phytic acid

MMFM had higher phytic acid content (278.2 mg/100 g) than control flour had (206 mg/100 g). The phytic acid increased in the experimental flour mix compared control flour mix. Similar findings have been reported by Chetana (2008) in different composite flour blends and chapathis. Phytates are thought to exhibit antioxidant properties by sequestering the iron before it can be involved in oxidation processes, thus, protecting against degenerative diseases. Furthermore, Pendelton (2009) reported that phytates can play a role in the reduction of digestion of starch, the glycaemic index of foods, cholesterol, and triglycerides production.

3.8 Antioxidant activity

The higher antioxidant content was observed in MMFM (93.3 mg/ 100 g) and a lower level in control flour (85.70 mg/100 g). Antioxidant activity increased with the incorporation of millet and other grains. Generally, the higher antioxidant activity of the mentioned fractions might be attributed to their contents of total polyphenols and especially flavonoids. However, according to Prior *et al.* (2005), many other compounds may contribute to the reaction and cause a false positive error. The main polyphenols in cereals are phenolic acids and tannins, whilst flavonoids are present in small quantities; they act as antioxidants and play many roles in the body's immune system defence (Chandrasekara and Shahidi, 2010; Devi *et al*., 2011).

4. Discussion

Present results revealed the moisture content decreased with addition of millet flour incorporation in MMFM. Chetana (2008) reported moisture content 11.50 per cent in composite mix I (pearl millet, sorghum, green gram and soybean) and 12.67 per cent in composite mix II (finger millet, sorghum, green gram, and soybean). In the present study, the moisture content might be low due to high fiber content, however low moisture content is a desirable attribute in increasing shelf life. Similary, the carbohydrate, energy of MMFM was decreased with addition of millets and other functional ingredients. Similarly, Roopa *et al.* (2003) had developed millet fortified dhokla mix and dhokla and reported that millet fortified dhokla energy decreased with an increased level of millet flour. Foxtail millet flour incorporated bread had an energy content of 257 kcal/100 g. In contrast, the control bread had an energy content of 266 kcal/100 g (Chhavi and Sarita, 2012). Energy is essential for activity, growth and rest (Deepa, 2009).

The proximate composition of MMFM was found the addition of millets and functional ingredients enhanced the ash, fat, crude fiber content at significant levels. In this study, the mineral content was increased in MMFM due to millet grains have a high content of minerals and ash itself showing the high mineral content in millet-based foods, our findings are aligned with Kruppaswamy (2013) and Geetu *et al.* (2003) for different millets based composite flours. About 0.6 to 3.5 g is an adequate daily intake for sodium (Manay and Shadaksharaswami, 2006). Sodium and potassium are important constituents of fluid present outside and within the cells. Proper concentration of these electrolytes is essential to maintain osmotic balance (Gopalan *et al*., 2004).

Hence, the estimation of RS becomes essential in food samples. RS content of MMFM was sigificanlty higher compared to control flour mix. Foods containing RS will have a moderate rate of digestion. The slow digestion of RS has implications for its use in controlled glucose release applications. The digestion over a 5 to 7 h period reduces the postprandial glycemia insulinemia. It can increase satiety (Raben *et al*., 1994; Reader *et al*., 1997). Also, it helps to manage meal-associated hyperglycaemia (Lehmann and Robin, 2007); this is particularly important for people who are at risk of suffering from type 2 diabetes. The dietary fiber and in *in vitro* protein content was high in MMFM. It is recommended that an increase of dietary fiber, particularly the soluble type, should be taken by T2D patients (Wood *et al*., 1990; Slavin, 2005). It is reasoned that soluble fiber reduces enzyme access to its substrates through viscosity effect. Slavin (2005) has concluded that fiber intake and obesity are inversely correlated, indicating that fiber is an important instrument in starch and cholesterol control. The hypoglycaemic effect of dietary fiber could be due to the delaying the starch hydrolysis, glucose absorption and also improvement in glucose utilization and insulin sensitivity in target tissues (Wahlang, 2018).

The antioxidant and antinutritional content of MMFM were found significantly high compared to control flour. The total phenolic compounds are one of the most effective natural antioxidants.The present results revealed the higher content of TPC of MMFM can be attributed to millets, pulses and fenugreek presence in the mix. Similar results were reported by Ashoush and Gadallah (2011) in sprouted mug incorporated composite flour mix. Flavonoid also plays a protective role against cancer, cardiovascular diseases and kidney diseases and *in vitro*, flavonoid has shown free radical scavenging activity and protection against reactive oxygen species (Shoba *et al*., 2010; Gu and Weng, 2001). The elevated antioxidant activity of MMFM might be due to the presence of the high amount of TPC and TFC.

In this study, phytic acid is a common storage form of phosphorus in seeds and is also considered as an antinutritional factor. The phytic acid content of MMFM 278.2 mg/100 g which is higher than the value reported by Abebe *et al.* (2007). Kruppaswamy (2013) reported that phytic acid has been shown to exhibit both á-glycosidase and á- amylase inhibition in a dose-dependent manner, also shown to possess antidiabetic activity against streptozotocin-nicotinamide induced diabetes (Type 2) rats.

5. Conclusion

The protein, fat, ash, and crude fibre, dietary fibre content was significantly higher in MMFM compared to wheat flour. The minerals, potassium, calcium, iron, and zinc content were significantly higher in composite flour than wheat flour. It would be an appropriate approach for providing balanced and required nutrients for diabetes. Blending techniques of nutrient-dense grains along with nutricereals will give required and balanced nutrients for reducing malnutrition and non-communicable diseases. Hence, it could be helpful in risk reduction of metabolic diseases.

Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

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