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RESEARCH ARTICLE

DEVELOPMENT OF MICRONUTRIENT ENRICHED COMPOSITE FLOUR WITH SELECTED GRAINS, PULSES, AND HERBS

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ABSTRACT

Most of the non-communicable diseases are directly related to the consumption of purified wheat flour. Therefore, composite flour mixtures can be introduced as a remedy. This study aimed to formulate a composite flour mixture comprising rice (*Oryza sativa*, variety Bg352), cowpea (*Vigna unguiculata*), horse gram (*Macrotyloma uniflorum*), black cumin (*Nigella sativa*), and ash plantain (*Musa paradisiaca*) pseudo stem. All the raw materials were cleaned and transformed into flour forms, and eight different composite flour mixtures were prepared according to *Taguchi*'s L8 orthogonal design. Eight samples of String hoppers, hoppers and pittu were prepared with eight different formulations. The sensory properties of prepared samples were evaluated using the five-point hedonic scale with thirty semi-trained panelists. The proximate composition, mineral profile, total phenolic content and antioxidant activity of the selected formulae were determined using standard analytical techniques. The colour, swelling index, initial water absorption capacity and bulk density of the final formulae were also evaluated. The highest rank for all the tested sensory attributes was obtained by the formula consisting of 82.93% *O. sativa*, 10.73% *V. unguiculata*, 3.90% *M. paradisiaca* pseudo stem, 1.95% *M. uniflorum,* and 4.88% *N. sativa* flour. The proximate results revealed that the sample with highest overall acceptability contains moisture (9.83%), ash (1.25%), total fat (1.28%), crude protein (10.55%), crude fiber (2.40%) and carbohydrate (70.25%). The results of the mineral profile of the sample with the highest overall acceptability for all sensory attributes revealed that sodium (1879.85 ppm), potassium (1276.20 ppm), calcium (2911.67 ppm), magnesium (8923.68 ppm), zinc (2.57 ppm), manganese(0.32 ppm), and iron (2.19 ppm). DPPH radical scavenging activity (0.0688 mg mL^{-1}) and gallic acid equivalent phenolic content (37.02 µg/mL) of the composite flour mixture were analyzed. The initial water absorption capacity (150.83), oil absorption capacity (1.99 g/g), swelling index (4.99 mL/g), bulk density (0.50 g/mL), and color (L*87.03, a*0.8, b*7.9, h 84.27) were recorded. The total plate count, yeast & mold count, *coliform* count, peroxide value and moisture content confirmed the product's safe consumption for two months. The developed composite flour mixture meets micronutrient requirements, and it can be used as a substitute for purified wheat flour for making string hoppers, hoppers, roti and pittu.

Keywords: Mineral composition, Physicochemical properties, Proximate composition, Sensory analysis and Scavenging activity

INTRODUCTION

Protein and micronutrient deficiency is a significant health problem among Sri Lankan children and pregnant mothers (Liyanage *et al.* 2014). The prevalence of iron, calcium, zinc, folate, and vitamin deficiency has been reported in Sri Lanka (Abeywickrama *et al.* 2018). Inadequate intake of proteins ultimately leads to malnutrition conditions, especially in children. Sri Lanka is among the top three South Asian countries in terms of insufficient intake of vitamins A and B12, calcium, and riboflavin (Abeywickrama *et al.* 2018). Rural and urban women suffer from malnutrition, with only 27% of protein requirement, deficiencies in vitamins A, C, and B, and minerals including calcium and iron (Weerasekara *et al*. 2020).

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Composite flour mixtures offer a versatile and sustainable approach to food formulation, and such formulae provide numerous benefits in food production by blending various types of flour like *T. aestivum*, maize, and legumes. Composite flour enhances nutritional value, providing a balanced mix of proteins, fibers, vitamins, and minerals, leading to healthier, more wholesome products. Further, composite flour enhances texture, flavour, and aroma, improving taste and sensory appeal (Chandra *et al.* 2015). Economically, composite flour can reduce production costs and dependency on single crops, increasing food security. Moreover, they aid in reducing the risk of diet-related diseases by improving the glycemic index and providing dietary diversity and several advantages of composite flour include better source of protein for human, promoting high-yielding native species, saving foreign exchange, enhancing domestic agriculture and acting as a rural income (Bugusu, Campanella, & Hamaker, 2001; Seibel, 2006; Iwe *et al.* 2016; Olaoye & Ade-Omowaye, 2011; Uukule, 2020; Noorfarahzilah *et al*. 2014). Composite flour is beneficial in developing countries due to its ability to minimize the necessity to import *Triticum aestivum* flour and improve the utilization of indigenous plant materials (Noorfarahzilah *et al.* 2014). Ijarotimi *et al.* (2021) evaluated composite flour based on *T. aestivum*, *Glycine max*, *Avena sativa* bran, and *O. sativa* bran. They concluded on its low glycemic index, higher inhibition potential of carbohydrate hydrolyzing enzyme, blood glucose level reduction and adequate nutritional qualities. A gluten-free composite flour mixture that is beneficial for people with gluten intolerance from rice, potato, cassava, millet and corn flour has been formulated by Quiñones *et al*. (2015). Udomkun *et al.* (2019) developed a macro and micronutrient-rich cereal-legume-based composite flour mixture (Udomkun *et al.* 2019), and a peanut-pearl millet flour mixture was formulated by Alhassan *et al*. (2019). There is a trending pattern to use protein-enriched composite flour mixtures made out of locally grown legume and cereal crops (Onyango, 2019; Okpalanma *et al.* 2020; Muldabekova *et al*. 2022). *T. aestivum* flour is considered nutritionally poor due to a lack of essential amino acids such as lysine and threonine (Siddiqi *et al*. 2020) and gluten consumers with gluten intolerance should avoid *T. aestivum* flour (Quiñones *et al*. 2015). Most non-communicable diseases are linked with food items produced with *T. aestivum* flour-based mixtures.

Introducing a composite flour mixture formulated using locally grown, underutilized, yet protein and micronutrient-rich materials for preparing hoppers, string hoppers, *roti*, and *pittu* will be a successful approach for introducing a healthy diet. Incorporation of legumes' incredibly underutilized nonpopular seeds into composite flour mixtures to produce popular food items will be an excellent strategy to compensate for dietary proteins and micronutrients. Cowpea, horse gram, black cumin, and plantain pseudo stems can be integrated into composite flour mixtures. Cowpea (*Vigna unguiculata)* is a rich and low-cost source of protein, which is considered a high-quality protein source with a unique protein profile of lysine, albumin, globulin, prolamins, and glutelin proteins. Horse gram (*Macrotyloma uniflorum)* is known as a poor man's meal and rich in albumin, globulin, resistant starch, oligosaccharides, dietary fiber, low glycemic, and non-digestible carbohydrates (Bhartiya *et al*. 2015; Herath *et al*. 2018). Plantain (*Musa paradisiaca*) pseudo stem (hereafter referred to as pseudo stem) mainly consists of lignocellulose and is usually used as a bio-fertilizer. The pseudo stem is rich in vitamin B6 which aids in producing haemoglobin and insulin. In traditional medicine, pseudo stem is used to treat high blood pressure, stomach disorders, diabetes, kidney and urinary bladder stones (Rajesh, 2017) Black cumin (*Nigella sativa*) is a rich source of proteins (Table 6), which contains many essential amino acids including glutamic acid, alanine, isoleucine, threonine, tyrosine, and lysine (Forouzanfar *et al.* 2014; Albakry *et al.* 2022).

Therefore, this study was conducted to formulate a micronutrient-enriched composite flour mixture that can be used for multipurpose food preparations and analyzed the physicochemical proximate, functional properties and shelf-life of the selected formulas.

MATERIALS AND METHODS

The raw materials were decided based on the available literature on physicochemical properties, proximate analysis, and health benefits. Rice (*Oryza sativa)*, cowpea (*Vigna unguiculata*), horse gram (*Macrotyloma uniflorum*), black cumin (*Nigella sativa),* and pseudo stems of ash plantain (*Musa paradisiaca)* were used as the ingredients of the composite flour mixture. The seeds of cowpea, horse gram, and black cumin were bought from the local market and rice variety *Bg352* (white pericarp) was obtained from a paddy field in Horagala, Matara. Ash plantain pseudo stems were collected from cultivation in Matara, Sri Lanka.

Preparation of rice, cowpea, horse gram flour and black cumin seed powder

Rice was soaked in water for 8 hours and dehydration was done at 60^{o}C for 6 hours. The cowpea and horse gram seeds were cleaned with fresh water and dehulled after roasting. Roasted rice, cowpea, and horse gram were ground and sieved separately by a 300-micron-sized mesh. Black cumin seeds were washed, sun-dried, ground, and sieved using a 300-micron sieve. The flour was packed in a triple-laminated package and stored at room temperature condition until further use (Figure 1).

Preparation of *M. paradisiaca* pseudo stem powder

Plantain pseudo stems were cleaned, cut into thin slices, and immediately dipped into a 1% (w/v) citric acid solution. Pseudo stem pieces were dried at 60^{o}C for 8 hrs and the powder was prepared after grinding and sieving by a 300-micron-sized mesh (Figure 1).

Product Formulation

A trial-and-error method was used to determine all flour types' lower and upper specification limits (Table 1).

Table 1: Lower and upper levels for formulation plan

Flour	Lower level (g)	Upper level (g)
Rice	83	85
Cowpea	9	11
Plantain	2	4
Horse gram	2	4
Black cumin	0.5	1.5

Figure 1: Raw materials used for the preparation of composite flour mixture. Upper panel from left to right: *Oryza sativa*, *Vigna unguiculata*, *Macrotyloma uniflorum*, *Nigella sativa* seeds and *Musa paradisiaca* pseudo stem and, Lower panel from left to right, the dried powder form of respective ingredients.

Rice, cowpea, horse gram, and black cumin flour were mixed to prepare eight composite flour mixtures according to *Taguchi's* L8 orthogonal array. (Rao *et al*. 2008) (Table 2).

Sensory evaluation

Hoppers, string hoppers, roti, and pittu samples were prepared and sensory evaluation was conducted by 30 semi-trained panellists at the Department of Food Science and Technology, University of Sri Jayewardenepura, Sri Lanka, to select the most appetizing formula for composite flour mixture. A five-point hedonic scale was used considering the sensory attributes – appearance, odour, texture, taste, aftertaste, and overall acceptability. Sensory data was analyzed by ANOVA using MINITAB 19 (Arnold, 2006). Sensory analysis was conducted for sensory set SS1 and sensory set SS2 samples separately, and the two best samples were selected according to sensory attributes. Two samples were again subjected to sensory analysis to select the most appropriate formula for the composite flour mixture.

Evaluation of physicochemical properties of the composite flour mixture

The best combination of composite flour mixture was analyzed for physicochemical properties.

Water and oil absorption capacity: Water and oil absorption capacity were determined by the method mentioned by Sosulski *et al*. (1976). An amount of 3.00 g composite flour mixture was added to a pre-weighed centrifugal tube with 10 mL of distilled water or virgin coconut oil. The sample was vortexed for 1 m and allowed to stand at room temperature for 30 min. Centrifugation technique was applied for 2 min at 4000 × g, the supernatant was discarded, and the weight of the sample was taken.

Color: A Colorimeter was used to obtain the composite flour mixture's L*, a*, b*, and h values. Readings were taken in triplicate in different areas of the sample.

Swelling index: Swelling index was determined according to the method mentioned by Marikkar *et al*. 2021. Composite flour was introduced up to the 10 mL mark of 100 mL measuring cylinder, and distilled water was added until the 50 mL mark. The top of the measuring cylinder was covered, and mixing was done by inverting the measuring cylinder. Inversion of suspension was repeated after 2 min and left for 8 min. The volume occupied by the sample was recorded.

Bulk density: Bulk density was determined according to the method mentioned by Marikkar *et al*. 2021. A portion of 5.00 g composite flour was introduced into a 50 mL measuring cylinder. The measuring cylinder was tapped on a surface until no visible decrease in volume was observed. Using the observed weight and volume, bulk density was calculated.

Determination of radical scavenging capacity and total phenolic content:

Accurately 5.00 g of composite flour mixture was weighed into a conical flask. A portion of

Table 2: Formulation plan based on Taguchi's L8 orthogonal array.

Trial	Code	Rice (g)	Cowpea (g)	Plantain (g)	Horse gram (g)	Black cumin (g)	Set number
1	101	83	9	2	2	0.5	SS1
2	201	83	9	4	2	1.5	
3	301	83	11	2	4	0.5	
4	401	83	11	4	4	1.5	
5	501	85	9	2	4	1.5	SS2
6	601	85	9	4	4	0.5	
7	701	85	11	2	2	1.5	
8	801	85	11	4	2	0.5	

50 mL methanol was added, and the sample was shaken using a shaker operated at 300 osc/min. The solution was centrifuged at 3200 rpm for 5 minutes to collect supernatant, filtered through Whatman filter paper, and stored in a refrigerator at 4-7 ^{0}C. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to measure the radical-scavenging capacity of the flour mixture (Blois, 1958). The total phenolic content was analyzed using AOAC official 2017.13 methods (Kupina *et al*. 2018).

Proximate analysis of composite flour mixture

The proximate analysis of the composite flour mixture was carried out according to standard methods. Moisture, ash, total fat, total carbohydrate, crude fiber, and crude protein contents were determined by AOAC official method 925.10 (McCleary, 2014), AOAC Official method 923.03 (Selvamani *et al*. 2009), AOAC official method 922.06 (Ali *et al*. 1997), phenol sulfuric acid method (Dubois *et al*. 1956), AOAC 2005,962.09 (Biswas *et al*. 2019), SLS 1549 PART 2: 2016, respectively.

Determination of micronutrients using Atomic Absorption Spectroscopic (AAS) method – Calcium, magnesium, sodium, potassium, zinc, manganese, and iron

The contents of calcium, magnesium, sodium, potassium, zinc, manganese, and iron were determined by AOAC Official method 942.05 (Biswas *et al*. 2019) using the Atomic Absorption Spectroscopic method.

Shelf-life evaluation of selected formula

The total plate count, yeast and mold count, *coliform* count, peroxide value, and moisture content were measured under room temperature conditions to determine the shelf-life of the composite flour mixture stored in triple laminated package. The protocols followed were SLS 516: part 1:1991 (Silva *et al*. 2016), SLS 516: part 2:1991 (Silva *et al*. 2016), AOAC Official 991.15 (Feng *et al*. 2002), AOAC Official 965.33 (Emil *et al*. 2010), AOAC Official 925.10 (McCleary, 2014), respectively.

RESULTS AND DISCUSSION

Sensory evaluation for selecting the best formula

According to the *Friedman* test results, there were significant difference ($p<0.05$) in appearance, odour, texture, taste, after taste, and overall acceptability for string hoppers, hoppers, pittu and roti samples in both sensory sets 1 (SS1) and 2 (SS2). The selected best samples were further analyzed using the *Wilcoxon-signed rank* test, and there were significant differences among all the tested parameters ($\alpha< 0.05$, $p<0.001$) in string hoppers, hoppers, *pittu* and *roti*. The final best formula was selected by using statistical analysis. Formula 801, which contained flour of *O. sativa* 85 g, *V. unguiculata* 11 g, *M. paradisiaca* 4 g, *M. uniflorum* 2 g, *N. sativa* 0.5 g in the mixture, recorded the best sensory acceptance among each two samples of four products.

Table 3: Mean values for the determination of final formulation with highest overall acceptability for considered sensory attributes.

	String hoppers		Hoppers		Pittu		Roti	
	SS1(101)	SS2(801)	SS1(101)	SS2(801)	SS1(101)	SS2(801)	SS1(101)	SS2(801)
AP	2.80±0.79^{a}	3.47±0.62^{b}	2.87±0.67^{a}	4.00±0.57^{b}	3.17±0.58^{a}	4.27±0.57^{b}	2.93±0.57^{a}	3.80±0.48^{b}
OD	2.87±0.56^{a}	4.00±0.52^{b}	3.23±0.62^{a}	4.30±0.64^{b}	3.43±0.67^{a}	4.33±0.75^{b}	3.00±0.58^{a}	3.90±0.54^{b}
TE	2.83±0.82^{a}	4.07±0.63^{b}	3.00±0.62^{a}	4.17±0.64^{b}	3.10±0.58^{a}	4.13±0.62^{b}	3.13±0.62^{a}	4.23±0.56^{b}
T	3.13±0.49^{a}	4.43±0.50^{b}	3.40±0.55^{a}	4.47±0.67^{b}	3.47±0.56^{a}	4.53±0.62^{b}	3.23±0.62^{a}	4.43±0.50^{b}
AT	3.23±0.88^{a}	4.37±0.60^{b}	3.30±0.64^{a}	4.60±0.49^{b}	3.37±0.75^{a}	4.67±0.60^{b}	3.37±0.60^{a}	4.57±0.56^{b}
OA	3.43±0.88^{a}	4.57±0.50^{b}	3.73±0.57^{a}	4.83±0.37^{b}	3.60±0.55^{a}	4.87±0.34^{b}	3.57±0.56^{a}	4.73±0.51^{b}

SS1: Sensory set 1, SS2: Sensory set 2, AP: Appearance, OD: Odour, TE: Texture, T: Taste, AT: After taste, OA: Overall acceptability. The values followed by the different superscript in the same row are significantly different at $p<0.05$, *t*-test

Physico-chemical properties of the composite flour mixture

Water absorption capacity, oil absorption capacity, color, swelling index, and bulk density were measured as physico-chemical parameters and data are shown in Table 4.

Table 4: Physico-chemical properties of the composite flour mixture

Physico-chemical property	Value
Water absorption capacity (%)	150.83±0.67
Oil absorption capacity (g/ g)	1.99±0.66
Swelling index (mL /g)	4.99±0.67
Bulk density (g/ mL)	0.50±0.044

The water absorption capacity of the developed composite flour mixture (150.8%) of the present study was lower than the reported water-holding capacity of wheat flour(140.00%) (Chandra, 2015). The water-holding capacity of other ingredients in the composite flour mixture, horse gram, cowpea and rice is 135.8-148.1%, 1.89%-2.15%, and 190-94%, respectively (Sreerama *et al.* 2008, 2012; Appiah *et al.* 2011; Chandra *et al.* 2015).The high water holding capacity in developed composite flour mixture is essential for improving texture, extending shelf-life, enhancing nutritional value, reducing crumbliness, promoting cost efficiency, enabling formulation flexibility, increasing yield, and reducing food waste in various food products. A high-water holding capacity is a clue to the existence of hydrophilic constituents. Further, protein contains both hydrophilic and hydrophobic parts that interact with water. Hence, protein concentration variations and conformational characteristics result in differences in water-holding capacity (Chandra *et al.* 2015). A high-water holding capacity is a quality parameter in the production process of meat analogues (Cornet, *et al.* 2021). The composite flour mixture would be a good basal mixture for such a product.

Color

L*, a*, b*, c*, and h correspond to the luminance or lightness component, greenness to redness, blueness to yellowness, chroma, and hue of the composite flour mixture as described by León *et al.* (2006). The data from three replicates recorded 87.03 ± 0.285, 0.8, 7.9 ± 0.113, 7.93 ± 0.131, and 84.27 ± 0.346 for L*, a*, b*, c*, and h*, respectively.

Oil absorption capacity.

The oil absorption capacity of the composite flour mixture was 1.9879±0.659, which is higher than the oil absorption capacity of wheat flour (0. 31± 0.002 g/g) (Onabanjo *et al.* 2020) (Table 5). The ability of food to bind with the oil is essential in food applications. The oil absorption capacity of rice flour varies between 0.556 g/g and 0.784 g/g (Awuchi *et al.* 2019). Furthermore, the oil absorption capacity of *V. unguiculata* and *M. uniflorum* is 2.9 g/g and 1.21 g/g, respectively (Abbey & Ibeh, 1988), which exceeds that of rice. The constituents other than rice flour has contributed to increasing the oil absorption capacity of the composite flour mixture.

Oil absorption capacity in flour mixtures is decisive in the food industry, influencing texture, flavor, and cost-efficiency. It makes crispy, less greasy textures in fried items, reduces the need for excessive oil, and enhances the retention of flavors (Najafi *el. al.* 2011). Additionally, it affects the moisture content in baked goods, impacting their shelf life and texture. In gluten-free or vegan recipes, it is essential for consistency. The

Table 5: Proximate composition (%) of the composite flour mixture

Component	*Composite	*T. aestivum*	*M. uniflorum*	*O. sativa*	*V. unguiculata*	*M. paradisi-aca*	*N. sativa*
Moisture	9.8303 ± 0.0922	12.48	9.28		11.88	10.74	4.56
Ash	1.2451 ± 0.0392	0.35		0.923	1.12	7.21	28.16
Total Fat	1.2797 ± 0.02	1.13	0.62	1.53	1.83	2.16	15.69
Crude Protein	10.5491± 0.119	7.98	21.73	8.00	22.85	13.11	18.44
Crude Fiber	2.4006 ± 0.0159	1.13		0.091	0.65	9.59	
Total carbohydrate	70.25± 4.49		57.24	89.46	61.67	57. 19	5.31

hydrophobic and hydrophilic parts of the proteins mainly contribute towards the oil absorption capacity of the flour mixture.

Swelling Index

The swelling index of the composite flour mixture was 4.9993±0.667, while it is 3.56 ± 0.13 for *T. aestivum* flour (Das *et al.* 2019). The swelling index is essential to control texture and hydration rates, optimise ingredient selection, ensure quality, and minimize costs. It is also critical in nutritional availability and finds applications in various industrial settings. Flours from various plant sources vary in amylose and amylopectin content, leading to differences in swelling capacity (Awuchi *et al.* 2019).

Bulk Density

The bulk density of the composite flour mixture was 0.5014±0.0387 g/ mL, which is comparatively lower than the *T. aestivum* flour (0.58±0.04 g/mL) (Das *et al*. 2019). Relatively low values of bulk density indicate increased energy content that can be derived from the flour (Tran *et al*. 2020). However, the bulk density of the constituents of the composite flour mixture, including *V. unguiculata, M. uniflorum,* and *N. sativa* flour, is reported as 0.71 g/cm³, 0.92 g/mL, and 0.46 g/mL, respectively (Appiah *et al.* 2011; Handa *et al.* 2017; Jan *et al*. 2019). Moisture content and the flour sample's particle size impact bulk density (Tran *et al*. 2020). Bulk density is essential to decide the packaging requirements of flour samples.

Chemical properties

Half maximal inhibitory concentration

The determined value for the half-maximal inhibitory concentration of the composite flour mixture was 0.0688 mg/mL. Higher the half-maximal inhibitory concentration values obtained for DPPH radical scavenging activity indicate the higher antioxidant content in the composite flour mixture. According to Jumina *et al*. (2019), a plant extract that has half maximal inhibitory concentration value lower than 100 mg/ mL is considered as very strong antioxidant activity.

Total phenolic content

The total phenolic content was 37.015 mg GAE/100 g in the composite flour mixture. The value for the pulse crops is between 38.6–542.7 mg GAE/100 g (Parikh *et al.* 2018). Phenolic compounds gain redox potential responsible for antioxidant activity. However, phenolic content varies with several extraneous factors such as amount of sugars, duration and method of extraction (Aryal *et al.* 2019).

Proximate Composition

The values of proximate composition of composite flour mixture were compared with the values of *T. aestivum* (Hussain *et al.* 2021), *V. unguiculata* (Odedeji *et al.* 2011), *O. sativa* (Hafeel *et al.* 2020), *M. uniflorum* (Aditya *et al.* 2019), *N. sativa* (Thilakarathna *et al.* 2018), *M. paradisiaca* (Ogofure & Emoghene, 2016) (Table 5). The total fat content was reduced, and crude protein content was increased in the composite flour mixture by adding other ingredients to *O. sativa*.

The developed composite flour mixture is rich in crude protein and crude fiber when compared to wheat flour.

Micronutrients Composition of composite flour mixture

The amount of calcium, magnesium, sodium, potassium, zinc, manganese and iron in the composite flour mixture was determined according to the atomic absorption spectroscopic method (Table 6)

Table 6: Micronutrients in the composite flour mixture using Atomic Absorption Spectroscopy (AAS) method.

Mineral	Amount (ppm)
Sodium (Na)	1879.8±112.8
Potassium (K)	1276.3±82.5
Calcium (Ca)	2911.7±198.6
Magnesium (Mg)	8923.7±679.7
Zinc (Zn)	2.5677±0.168
Manganese (Mn)	0.3194±0.033
Iron (Fe)	2.1921±0.298

Shelf-life evaluation

Total plate count, Yeast and mold count, and Coliform counts were observed weekly for two months (Table 7). No Coliform was detected throughout the testing period. According to the SLS guidelines, the acceptable total plate count for cereal-based products is 10^5 and 10^6 cfu/g. The observed total plate count for the composite flour mixture fluctuates within the safe limit. The maximum Coliform count for cereals and legume-based flour mixtures is 1×10^1 cfu/g mixture (Food Act, 1980). The observed yeast and mold counts (too few to count) were lower than the standard limit of yeast and mold count recommendation: 1×10^3 per gram of flour mixture (Food Act, 1980). Therefore, the product is microbiologically safe regarding yeast and mold count and Coliform count. Samples were packed in triple laminated pouches, and storage was maintained at room temperature. According to the results, composite flour mixture packed in triple laminated packaging is microbiologically safe for two months of shelf -life.

Table 7: Determination of total plate count and yeast & mold count of the composite flour mixture*

Time (Week)	Dilution	Total Plate Count (CFU/g)	Yeast & Mold Count (CFU/g)
7	10^{-1}	N.D.	N.D.
	10^{-2}	N.D.	TFTC
	10^{-3}	N.D.	N.D.
	10^{-4}	N.D	N.D
8	10^{-1}	TFTC	TFTC
	10^{-2}	TFTC	TFTC
	10^{-3}	N.D.	TFTC
	10^{-4}	N.D.	N.D.

The first six weeks were not included since values were ND, N.D. : Not detected
TFTC: Too few to count

Analyzing peroxide values

Peroxides are considered precursors to breakdown products that can cause a rancid taste._Peroxide value increased with time due to developed rancidity (Table 8). Storage conditions, as well as heat and light conditions, may also be responsible for raising the acid value. The breakdown of unsaturated fatty acids in the mixture and the production of secondary oxidation products ultimately lead to an increase in peroxide. Similar results were reported by Shazadi *et al*. 2005 revealed that the composite flour mixture consisting of wheat, lentil, chickpea and guar gum had peroxide value of 0.99 (mgEq/kg) for 60 days of the storage period.

Table 8: Peroxide value analysis for composite flour mixture

Sample	Peroxide Value (mgEq/kg)	Moisture Content (%)
Initial	0.6649 ± 0.0298	9.8303 ± 0.0922
After two months	0.9136 ± 0.0298	10.1321± 0.0107

Analyzing moisture content

Even though the moisture content of the product increased after two months, the microbial count fluctuated between the standard limits for the product. The product was safe to consume. This value meets the specification of not more than 15.5% moisture in flour blends (Tangariya *et al*. 2018).

CONCLUSIONS

The present study introduces a novel composite flour mixture enriched with micronutrients, formulated from local underutilized food crops for versatile applications. The selected best formulation comprises 82.93% rice, 10.73 % cowpea, 3.90 % horse gram, 1.95 % ash plantain pseudo stem, and 0.49% g black cumin flour. The initial water-holding capacity of the composite flour mixture was 150.83%. The oil absorption capacity, swelling index, and bulk density were recorded as 1.99 g/g, 4.99 mL/g, and 0.50 g/mL, respectively. Color measurements were as follows: L* 87.03, a* 0.8, b* 7.9, and h 84.27. DPPH radical scavenging activity (half maximal inhibitory concentration) and gallic acid equivalent phenolic content were 0.0688 mg/ml and 37.02 µg/ml, respectively. The composite flour mixture had the following proximate composition: 9.83% moisture content, 1.25% ash, 1.29% total fat, 10.55% crude protein, 2.40% crude fiber, and 70.25% carbohydrate, while it contained 1879.85 ppm sodium, 1276.2 ppm potassium, 2911.67 ppm calcium, 8923.68 ppm magnesium, 2.57 ppm zinc, 0.32 ppm manganese, and 2.19 ppm iron. Based on the results of yeast and mold count, total plate count, *coliform* count, moisture content, and peroxide content analyses, the composite flour mixture is microbiologically safe within established limits for a duration of two months. This study presents a gluten-free, nutrient-enriched composite flour mixture, utilizing local crops. The flour mixture is physiochemically good for diverse food products. This development addresses gluten intolerance, non-communicable disease concerns, and potential malnutrition, while reducing dependence on imported wheat flour, leading to cost savings. Future research may involve consumer behavior analysis through a consumer panel and market analysis.

AUTHOR CONTRIBUTION

DRR and MAJW designed the study. DRR performed the experiments. DRR analysed the data. DRR wrote the paper. MAJW commented on the manuscript.

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