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CONTENTS

Physico-chemical and anti-nutritional properties of predigested composite flour mix from corn and green gram

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ABSTRACT: Mung bean (*Vigna radiata*), commonly called green gram, is a nutrient-rich legume from the Fabaceae family, widely consumed for its health benefits and versatility in savoury and sweet dishes. It is a valuable source of plant-based protein, dietary fibre, essential vitamins, and minerals, making it an integral part of balanced diets globally. Corn (*Zea mays*), a gluten-free cereal staple, is equally celebrated for its high content of carbohydrates, protein, and essential micronutrients such as magnesium, phosphorus, and vitamin B complexes. Despite their inherent nutritive properties, mung bean and corn exhibit anti-nutritional factors such as phytates and tannins that can reduce nutrient absorption and bioavailability. Enhancing these grains' nutritional profiles while addressing these limitations is crucial for their effective incorporation into health-focused diets.This study utilized germination and lactic acid fermentation as innovative processing techniques to improve the digestibility and bioavailability of nutrients in mung bean and corn. These processes have been shown to reduce anti-nutritional compounds, enrich grains with beneficial bioactive compounds, and enhance their overall nutritional quality. Nine composite flours were developed by blending different ratios of germinated and fermented flours of mung bean and corn. The physico-chemical properties, such as moisture content, nutrient composition, and texture, were analyzed alongside anti-nutritional factors to evaluate the suitability of these flours. The findings underline the significant potential of predigested flours for creating value-added recipes that cater to modern dietary needs, including high-protein, high-fiber, and gluten-free food products. These flours could be pivotal in addressing nutritional deficiencies, improving gut health, and developing functional foods to support better health outcomes.

Keywords: Composite flour, germination, fermentation, corn, mungbean

Composite flour is a blend of flours produced from cereals, roots, tubers, and legumes, with or without wheat flour. It promotes using indigenous agricultural products such as flour, and accepting these native crops would increase their usage via processing and value addition (Bello *et al.*, 2022). Eating whole grain cereals, legumes, and their products, including biscuits, prevents nutrition-related non-communicable diseases (Irondi *et al.*, 2024).

Green gram (*Vigna radiata*) is a grain legume that matures in 70–90 days and is also known as mungbean or golden gram in some places worldwide. It is commonly cultivated in the tropics and subtropics. It is consumed as cooked beans, broth, or pancakes and is an essential dietary source for people. Green gram seeds are high in protein (20.97–31.32%), and their carbohydrates are easier to digest, resulting in less flatulence in humans than other legumes (Nair *et al.*, 2013). It is a healthy legume that is widely consumed in South East Asia. It is well-known for having a high digestibility and not causing flatulence, which is frequently associated with many legumes. Legumes have dietary fibre content ranging from 8 to 27.5% and soluble fibre content ranging from 3.3% to 13.8% (Eashwarage, 2017).

Cereals are an excellent source of vitamins and minerals, including fat-soluble vitamin E, an essential antioxidant. The cereal grains are an easy protein source as required by Recommended Daily Allowance (RDA) (Khatkar, 2005).Across the world, nutritional meals are produced from grains like wheat, rice, maize, oats, barley, rye, sorghum, millet, buckwheat, and quinoa, and this are then eaten whole, polished, or ground(Ding and Feng, 2025).Maize is one of the most widely cultivated plants in the world and the third most important cereal after rice and wheat (GwirtzandGarcia-Casal, 2014). In addition, corn is a very interesting and nutritionally edible plant due to its richness in protein and certain minerals, carbohydrates and vitamins

(Shah *et al.*, 2011). However, anti-nutritional compounds can affect the digestibility of proteins and other nutrients (Gnanwa *et al.*, 2021). Corn flour develops and processes a vast range of foods and goods. "Food professionals have investigated the potential of various food ingredients/crops as well as appropriate technological processes to develop gluten-free products, but the quality of biscuits from blends of maize and soybean flour has not been assessed" (Okereke,2023).

Germination is a simple, inexpensive, and environmentally friendly method of producing plant foods with functional properties. It enhances the nutritional and medicinal properties of plant foods by reducing antinutrients and increasing the accumulation of antioxidants, flavonoids, phenolic acids, and vitamins, thereby increasing the value of grains (Durovic *et al.*, 2021). The best protein sources are legumes, starch, vitamins, and minerals. They serve as meat substitutes or alternatives that give the vegetarian diet all the nutrients it needs due to their excellent nutritional value (Chaturvedi and Chakraborty, 2021). A final product with enhanced nutritional content, safety, stability, and sensory qualities may be produced through carefully regulated grain sprouting and fermentation processes (Wu and Xu, 2019).

Fermentation is one of the earliest methods used to preserve food. Native microbiota, such as moulds, aerobic spore formers, lactic acid bacteria (LAB), and enterobacteria, compete for nutritional deposits in cereal and pseudocereal grains. Many species of Lactobacillus are well-nourished by cereals and pseudocereals (Wronkowska *et al.*, 2023). Fermentation can convert nutrients such as carbohydrates and proteins into usable end products and increase the contents of essential amino acids, proteins, and minerals in foods . Lactobacillus fermentation has been reported to be one of the most cost-effective methods to maintain or improve food products' nutrition, organoleptic, safety, and shelf-life (Kwaw *et al.*, 2018). It can lead to partial or complete degradation of anti-nutritional factors, thereby improving protein quality and bioavailability of minerals (Gobbetti *et al.*,2019). Although lactic acid bacteria (LAB) have been widely used in the food fermentation industry, studies on the effect of *Lactobacillus* fermentation on the nutritional value of potato flour have not been reported. Since most LAB cannot directly degrade native starch, fermentation of starch-based foods is inefficient. The current study aimed to standardize the procedure for preparing a predigested mixture of green gram and corn and evaluating its quality.

MATERIALS AND METHODS

Pure culture of *Lactobacillus acidophilus* ATCC was procured from the Institute of Microbial Technology, Chandigarh, India or ATCC. The culture was maintained on slants and sub-cultured after every 30 days. Corn and green gram were procured from the Genetics and Plant Breeding Department, CCS Haryana Agricultural University, Hisar. We purchased other ingredients from the nearby market.

Treatments

I. Germination: Grains were hygienist separately with 0.1% sodium hypochlorite (Grains: NaOCl ratio, 1:5 w/v) for 30 minutes and rinsed with distilled water. They were then soaked separately in distilled water (1:5 w/v grain to distilled water ratio) at room temperature and left overnight—hydrated grains germinated in the dark. The maximum time of germination was ûxed by achieving 95% sprout grains.

II. Fermentation: After cleaning, the grains were left to soak for 12 hours at room temperature in distilled water. The soaked grains were drained and dispersed in distilled water to make a slurry. *Lactobacillus acidophilus* (10^7 cells/ml) subjected the slurry to lactic acid fermentation. The germinated and fermented grains were dried at 55±5°C and kept in airtight containers.

III. Preparation of composite flour: Refined wheat flour (WF), mungbean flour (MF), germinated and fermented mungbean flour (GMF and FMF), corn flour, and germinated and fermented corn flour (GCF and FCF) were used to prepare composite flour. Nine different kinds of composite flour were made. One was prepared from whole wheat flour (WF) and three composites flours prepared from WF: MF: CF of 37.5:37.5:25 (C1),25:25:50 of (C2) and 12.5:12.5:75 (C3), three germinated composite flour consists WF:GMF: GCF of 37.5:37.5:25 (G4), 25:25:50 of (G5) and 12.5:12.5:75 (G6) and fermented composite flour consists WF: FMF: FCF of 37.5:37.5:25 (F7), 25:25:50 of (F8) and 12.5:12.5:75 (F9). The composite flours were passed through a 60 mesh size sieve for uniform mixing.

Proximate composition

Samples were evaluated for moisture content using a hot air oven, protein using the micro-Kjeldhal method with the KELPLUS Nitrogen estimation system, fat using the automatic SOCS plus Solvent extraction system, crude fibre using the automatic Fibre-Plus apparatus, and crude ash content using the standard AOAC method (2005). The Hulme and Narain(1931) methods calculated the total and reducing sugars. The sample's available iron was extracted (Rao *et al.*, 1978)., and available zinc was extracted(Kim and Zemel, 1986). The amount of calcium was estimated using the AOAC (2005) standard procedure. Phytic acid was measured using the Haughand Lantzsch (1983) technique. Total polyphenols were extracted with the help of Singh and Jambunathan (1981).

The carbohydrate content was determined using the difference technique AOAC (2005) on a dry basis using the following formula: Total carbohydrates = 100 – (crude fat + crude protein + ash +crude fibre)

Using the following formula, the energy was determined using the factorial technique by multiplying the sample's protein, carbohydrate, and fat contents by 4, 4, and 9, respectively.

Energy (K cal/100g) = 4.0 x protein (%) + 4.0 x carbohydrate (%) + 9.0 x fat (%).

Sensory evaluation- Chapattis were prepared from composite flours and evaluated for sensory parameters using a 9-point hedonic scale.

Statistical analysis: The data in this study were analyzed using SAS software and analysis of variance (ANOVA) procedures, following a completely randomized design.

RESULTS AND DISCUSSION

The present study tried to develop composite flour from predigested grain (Mungbean and Corn). The composite flours' functional, chemical, and nutritional characteristics were examined. Nine types of composite flours (Figure 1.) were prepared by mixing various ratios of refined wheat flour (RF), mungbean flour (MF), germinated mungbean flour (GMF), fermented mungbean flour (FMF), corn flour (CF), germinated corn flour (GCF), and fermented corn flour (FCF).

Nutritional composition of RF, MF, GMF, FMF, CF, GCF and GCF

Proximate composition

Control samples (refined wheat flour, MF and CF), germinated (GMF and GCF), and fermented flour (FMF and FCF) were analyzed for proximate composition (Table 1).

There was a significant difference ($P<0.05$) in the proximate composition of fermented, germinated, and refined wheat flours. The moisture content of a food indicates dry matter in that food (Adedeji*et al.*, 2014). Crude protein of GCF (20.52%) and crude fibre of GMF (17.03%) were the highest among all (Table 1). Fibre is essential to the diet that regulates

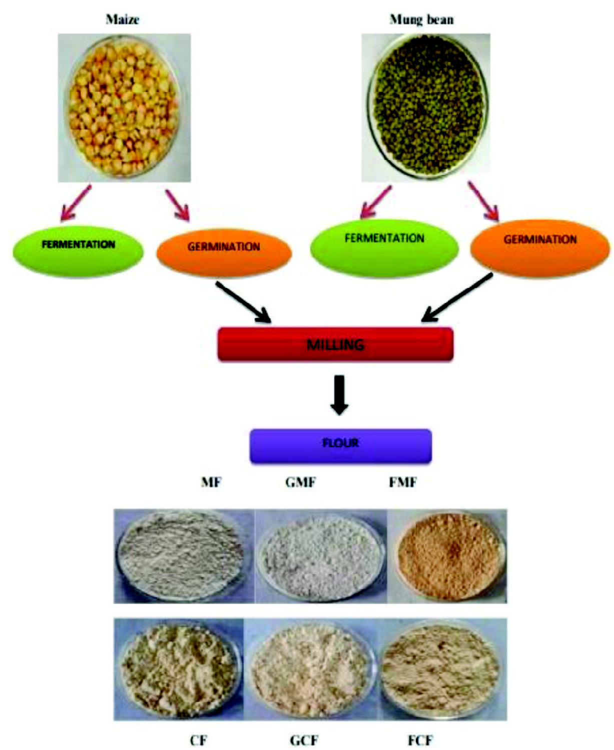

Fig. 1: Composite flour prepared by predigested Mungbean and Corn

bowel movement and weight. The increase in flour's crude fibre and ash contents is supported by Shah *et al.* (2011) and Devi *et al.* (2015), respectively.After sprouting, mung beans have increased antioxidant activity and are a good source of fibre, protein, vitamins, minerals and potential anti-cancer properties (Yadav, 2024).

CF, GCE and FCF contained (7.79, 7.90 and 6.49) moisture content; (1.12, 0.67 and 1.83%) ash; (5.93, 8.46 and 6.51 %) crude protein; (4.69, 4.42 and 4.41%) crude fat; (3.00, 3.03 and 3.16%) crude fibre and (84.98, 85.25 and 84.63%) carbohydrates respectively (Table 4.1). Similar findings in fermented corn flour (11.69% moisture, 7.63% crude protein, 4% crude fat, 1.62% ash, and 86.74% carbohydrate, respectively) were reported (Rahmawati *et al.*, 2018). The results show that GMF contained maximum crude protein and ash, whereas GCF contained maximum crude fat and total carbohydrates. Xu *et al.* (2020) reported that the GMF, which had high amounts of protein, ash, total sugar, lipid and fibre, could mix with the RF to produce gluten-free products with enhancement of the texture and nutritional quality of the final product. Pulses are a great protein, fibre, vitamins, and minerals source. Two examples of pulses are green gram and gram. The effect of adding sprouted green gram and sprouted gram flour to biscuits made with refined flour. When sprouted flour is added, the biscuits' protein content rises, but their fat and carbohydrate contents fall (Ding and Feng, 2025).Disrupting these interactions during fermentation and germination releases nutrients and phytochemicals, making them available to digest enzymes (Nkhata *et al.*, 2018). As expected, the fermentation of legumes degrades carbohydrates while increasing the concentration of soluble carbohydrates more easily absorbed by the gut (Adebo *et al.*, 2022). The composition of mungbean and corn changed after germination and fermentation.

Table 2:Mineral content in different composite flours

Flour	Iron(mg/100g)	Zinc(mg/100g)	Calcium(mg/100g)
WF	1.40±0.03[e]	1.39±0.01[e]	51.66±23.62[cd]
MF	2.15±0.07[b]	2.20±0.02[bc]	83.66±8.50[ab]
GMF	1.95±0.08[c]	1.86±0.05[d]	89.00±15.52[a]
FMF	2.40±0.07[a]	2.40±0.14[a]	91.33±3.21[a]
CF	2.07±0.05[b]	2.15±0.08[c]	31.66±7.63[d]
GCF	1.68±0.06[d]	1.73±0.20[d]	37.33±6.80[d]
FCF	2.34±0.02[a]	2.36±0.05[ab]	61.66±17.38[bc]
CD	0.1	0.18	23.76

Values are mean ± SE of three independent determinations.

Acidity and pH

The pH was an essential factor influencing overall qualities like colour, flavour and texture (Starowicz

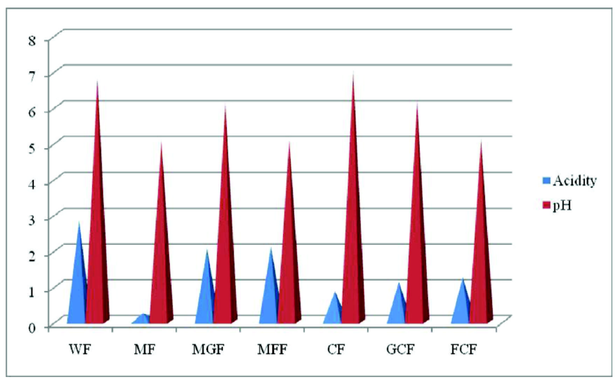

Fig. 2: Acidity and pH of flour

Table 1:Proximate composition of different germinated and fermented flour

Flour	Moisture (%)	Ash (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Total carbohydrates (%)	Energy (kcal/100g)
WF	12.55±1.78[a]	1.26±0.78[c]	13.23±3.97[ab]	0.75±0.79[bc]	11.83±0.20[a]	72.91±3.75[a]	323.23±26.65[a]
MF	8.65±5.25[a]	3.75±1.85[a]	11.52±3.26[b]	1.04±0.47[c]	16.53±5.24[a]	66.93±5.29[a]	321.45±23.65[a]
GMF	10.17±7.50[a]	3.87±0.08[a]	12.99±3.25[ab]	1.01±0.40[c]	17.03±5.74[a]	65.08±2.78[a]	358.37±51.85[a]
FMF	8.43±4.28[a]	3.27±1.19[ab]	15.17±5.26[ab]	1.09±1.02[c]	8.50±12.05[a]	71.95±6.48[a]	356.61±64.67[a]
CF	10.70±4.34[a]	1.12±0.69[c]	17.51±3.15[ab]	3.52±1.58[ab]	14.13±14.85[a]	63.70±12.50[a]	376.48±17.90[a]
GCF	10.75±4.35[a]	1.69±0.45[bc]	20.52±8.91[a]	4.92±3.97[a]	10.33±0.11[a]	62.51±12.81[a]	366.16±6.98[a]
FCF	7.90±2.68[b]	1.83±0.17[bc]	17.22±3.72[ab]	4.08±1.75 [a]	11.73±0.37[a]	65.12±2.41[a]	351.38±5.35[a]
CD	8.12	1.64	8.58	3.1	13.67	13.54	63.56

Values are mean ± of three independent determinations

WF = Refined wheat flour, MF= Mungbean flour, GMF= Germinated mungbean flour FMF=Fermented mungbean flour CF=Corn flour, GCF= Germinated corn flour, FCF = Fermented corn flour and Zielinski, 2019). Figure2 shows a significant (P<0.05) difference in pH and acidity among the various flours.

FMF contained higher acidity than MF and FCF, but GMF contained higher pH than MF and FCF. FCF is more acidic than CF and GCF. Fermented cassava flour with L. plantarum strain (LP) had the highest total titratable acidity, while commercial cassava flour had the lowest (Isa *et al.*, 2021).

Fermented flour may have a pH drop as a result of the fermentation process producing different organic compounds such as lactic, acetic acid, and ethanol, or a rise in acidity due to an increase in the concentration of fatty acids, phosphoric acids, H+, and carboxy groups of protein amino acid (Offiah *et al.*, 2017).

Minerals

All composite flours were analyzed for minerals (Table 2). There was a significant difference (P<0.05) found among the minerals (Fe, Zn, and Ca) observed in each flour. Similar results in the calcium content of mungbean were also studied (Ohanenye *et al.*, 2020).

Essential nutrients, including potassium, phosphorus, zinc, calcium, iron, thiamine, niacin, vitamin B6, and folate, are abundant in corn flour(Arshad *et al.*, 2023). FMF and FCF stand out with the highest iron content, surpassing 2.3 mg/ 100g, while FMF also ranks highest in zinc and calcium content. This suggests that fermentation may play a positive role in enhancing mineral bioavailability. Mungbean fermentation or germination increased the accessibility of in vitro iron, zinc, and calcium (Nkhata *et al.*, 2018). Calcium, iron, and zinc concentrations enhanced when grass peas were fermented with lactic acid (Adebo *et al.*, 2022).In the present experiment, FCF contained maximum iron, zinc and calcium (Table 2). During fermentation, dry matter is lost whenever bacteria break down protein and carbohydrates, which increases mineral concentration (Nkhata *et al.*, 2018). The fermentation process improved mineral availability due to the breakdown of oxalates and phytase.

Protein bioavailability, particularly that of thiamine, iron, and calcium, increases dramatically during germination. Additionally, it helps to increase the in vitro digestibility of starch and protein in dehulled and germinated green grams, cowpeas, lentils, and chickpeas (Starowicz and Zielinski, 2019).

Sugars

Figure 3 shows a significant difference (P<0.05) in total and non-reducing sugar throughout all flours. The results showed that FCF contained higher non-soluble sugar than other flour.

MF, GMF, and FMF contained 4.40, 11.58, and 4.67 g/100 g total soluble sugar and 2.71, 3.99, and 3.75 g/100g non-reducing sugar. Compared to MF, FMF, and GMF, GMF had a higher total soluble and non-reducing sugar content. Germination significantly increased the amount of sugar in green gram (Chandra *et al.*, 2015; Nkhata *et al.*, 2018; Yadav 2024).

In the present study, the total sugar of CF, GCF, and FCF was 12.05, 16.04, and 13.06 g/100g, respectively. The enzymatic breakdown of starch in simple sugars during germination results in a significant increase in both total and non-reducing sugar. When monosaccharides hydrolyzed starch, sugar concentration in pulses increased (Starowicz and Zielinski, 2019). Germination of mung beans increased total sugars by germination (Chandra *et al.*, 2015; Yadav 2024).

Anti-nutritional factors

Among composite flours, GMF contained higher phytic acid and polyphenols.Phytic acid and polyphenol content increased in germinated mungbean (Chandra *et al.*, 2015; Yadav 2024). For

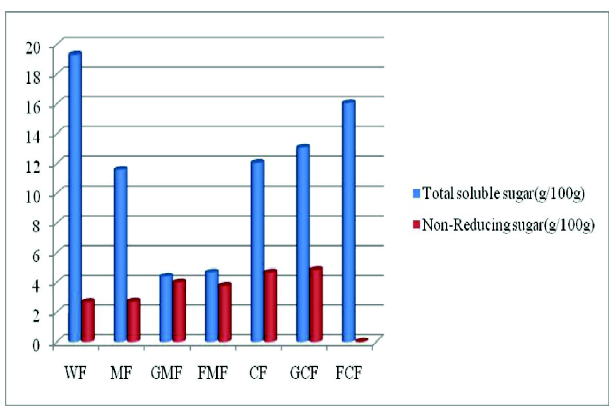

Fig. 3: Total soluble sugar and non-reducing sugar in flour

Table 3: Phytic acid and polyphenol content of different composite flours

Flour	Phytic acid (mg/100 g)	Polyphenols (mg/100 g)
WF	273.33±103.09[bcd]	369.33±129.26[a]
MF	401.73±140.24[a]	313.86±72.71[a]
GMF	416.40±58.62[b]	300.80±38.06[ab]
FMF	363.73±127.64[bc]	249.33±79.05[b]
CF	152.86±8.35[d]	55.65±13.67[c]
GCF	253.73±22.54[cd]	51.09±16.07[c]
FCF	218.26±56.48[cd]	52.10±17.11[c]
CD	153.52	115.47

Values are mean ± SE of three independent determinations

all flours, there was a significant (P<0.05) difference between the anti-nutritional (phytic acid and polyphenols) (Table 3).

Results indicated that GMF contained higher phytic acid and polyphenols than fermented treatments.During germination, the total phenolic contents of barley and mung beans increased significantly (Chandra *et al.*, 2015; Yadav 2024; Gupta and Bhatt 2024).The process of fermentation results in a significant decrease in phytic acid, polyphenols, and other antinutrients because the fermented dough has a low pH, which is optimal for phytase activity.Lectins and other proteinaceous anti-nutritional agents are affected by microbial proteolysis. The disulfide bonds should be broken to cause structural disruption, which is necessary for proteolysis. Several microbes have shown glutathione reductase activity. During the fermentation of legumes, thiol exchange processes catalyzed by glutathione are necessary to break down lectins (Arshad *et al.*, 2023).

The nutritional value of foods based on cereals is increased by the fermentation of cereal flour, which breaks down anti-nutritional factors. Fermented cereals with reduced tannin and phytic acid concentrations have more bioavailable minerals, which boosts the food's nutritional value (Adebo *et al.*, 2022). Increases in total phenolics and flavonoids were found in fermented cereals due to higher enzyme activity, and there was a positive correlation between total phenolics and antioxidant activity was also observed in previous studies (Saharan *et al.*, 2017).A higher amount of phytate consumption leads to a lack of certain minerals, such as zinc, magnesium, calcium, copper, and manganese, it hurts the minerals' bioavailability (Nkhata *et al.*, 2018). Sensory value enhanced due to reduced anti-nutritional factor after fermentation increases mineral availability.

CONCLUSION

Refined wheat flour remains a staple in many cereal-based recipes; however, enhancing its nutritional profile by incorporating germinated and fermented cereal and pulse flours presents a promising opportunity. The significant increase in protein, calcium, iron, and zinc observed in fermented mung bean and corn flours highlights their potential to address nutrient deficiencies and promote health benefits. Chapattis prepared using specific blended flours (C2, C3, G5, and F9) exhibited desirable sensory characteristics, demonstrating the feasibility of incorporating composite flours into daily diets. Looking ahead, the application of composite flours holds significant promise in the development of diverse value-added products, such as protein-enriched breads, gluten-free baked goods, and nutritionally fortified snacks. Future research could explore optimizing blending ratios, extending shelf life, and assessing consumer preferences further to enhance these innovative flours versatility and market potential. By leveraging these advancements, composite flours can address global nutrition challenges and foster functional foods tailored to modern health-focused lifestyles.

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