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CONTENTS

Evaluation of *in vitro* digestibility, functional and sensory characteristics of pre-digested corn and mungbean composite flour

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ABSTRACT: The study of nutritional enrichment and functional diversification in food products has led to the exploration of composite flours, which blend wheat flour with other nutrient-dense flours. Wheat provides essential carbohydrates to fuel the body. Corn is ideal for people with gluten sensitivities or celiac disease. It is rich in lutein and zeaxanthin, antioxidants that protect against macular degeneration. Corn is high in fiber, which improves digestion and supports a healthy gut. Mungbeans are great plant-based protein source for muscle repair and growth. It helps to regulate blood sugar levels due to its fiber and protein. It contains flavonoids and phenolic acids that combat oxidative stress and inflammation. In this investigation, refined wheat flour, corn, and green grams were combined to produce nine different types of composite flours. Corn and green gram composite flour were prepared by predigestion using germination and fermentation to enhance the nutritive value. Pre-digested composite flours had improved protein, dietary fibre, *in vitro* starch, *in vitro*proteindigestibility,rheological and sensory properties. water absorption capacity, swelling indexandpasting properties. These findings support the potential for developing nutritious, functional, and sensory-pleasing bakery products using pre-digested flour.

Keywords:Composite flour, corn,*in vitro* starch digestibility, *in vitro* protein digestibility,mungbean

Determining the mechanical behaviour of the wheat flour dough represents a significant challenge for the baking industry. The increasing unpredictability of wheat quality due to changes in the environment or sourcing diversification makes it more and more crucial to carefully assess the popular rheological models in a review to ascertain their ability to support industrial decision-making. Interestingly, most of the dough's rheological characteristics are established during the mixing phase, which is the initial procedure. Mixing produces the mechanical energy and deformation required to hydrate and distribute the flour's constituent ingredients uniformly and develop the network of gluten proteins (Della Valle *et al.,* 2022).

Beans are a nutritious source of dietary fibre, proteins, and complex carbohydrates. However, they can have a slightly acidic pH level and are rich in minerals and vitamins, which can be used as potential energy sources. Mung beans (*Vigna radiata L.*), which are comparable in nutritional content to red beans and soybeans (*Glycine max L. Merrill*) and are a rich source of vitamins, minerals, and essential amino acids, are an often utilized component for fortification (Tjokrokusumo *et al*., 2019). Corn has sufficient fibre in addition to protein and carbohydrates to be utilized as a raw material for diets containing high fibre (Viswanathan *et al*., 2019). Grain enzymatic activity is increased during germination, breaking down materials such as proteins and carbohydrates. As a result, germinated legumes can be successfully employed in food that requires enzyme activity. Some enzymes are naturally lacking in wheat, so adding them to wheat flour improves the dough's rheological characteristics and the quality of baked goods (Codina *et al.,* 2019).

The germination process improves some grains' organoleptic qualities, which breaks down high-molecular-weight polymers to form bio-functional molecules and softens texture and flavour (Banerjee *et al.,* 2020). LAB fermentation is recognized as a reasonably natural process that complies with consumer expectations of "green safety" and "sustainability. " Legume proteins fermented by LAB have greater functional and physical qualities, increased sensory quality and nutritional characteristics. Thus, better suited for specialized food products (Jena and Panda, 2023).

The main objective of the current investigation was to evaluate the impact of germination and fermentation on composite flour's functional characteristics and sensory 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

For germination, grains were 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 (Siddiqui *et al.,* 2019).
For fermentation:After cleaning, the grains were left to soak for 12 hours at room temperature in distilled water. *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 (Wronkowska*et al*., 2018).

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)are utilized in the production of composite flour. Nine varieties of composite flour were produced. 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.

In vitro protein and starch digestibility

Hamaker *et al*. (1986) used a modified method of measuring *in vitro* protein digestibility. They evaluated the *in vitro* digestibility of starch using pancreatic amylase (Singh *et al.,* 1981).

Functional properties

The Singh and Singh (1991) method estimated the water absorption capacity. The flour's swelling power was estimated using the method (Subramanian *et al*.,1986). Using the Rapid-Visco Analyzer (RVA-Super 3) Newport Scientific Australia, flour (300μ) was evaluated for various pasting characteristics, including peak viscosity, peak time, breakdown, final viscosity, setback, and pasting temperature.

Sensory evaluation

A panel of ten semitrained or untrained judges used a 9-point hedonic scale to evaluate the biscuit samples prepared from the processed and unprocessed mungbean and corn's sensory qualities just after they were prepared.

Statistical analysis

A suitable statistical analysis of the data will be performed. The data used in this study was examined using a completely randomized design and subjected to analysis of variance (ANOVA) procedures. During a three-month storage period, the critical difference value at the 5% level was used to compare the various treatments. SAS software was used to examine the data statistically.

RESULTS AND DISCUSSION

In vitro digestibility

As Table 1 shows, a significant difference was recorded between all flours. FCF had higher *in vitro* protein digestibility and *in vitro* starch digestibility.

Refined wheat flour contained 37.26% *vitro* protein digestibility and 35.33% *in vitro* starch digestibility. MF, GMF, and FMF contained 57.22, 63.02, and 68.91 *in vitro* protein digestibility and 24.80, 27.73, and 29.20 *in vitro* starch digestibility, respectively, in Table 1. Similarly, Onwurafor*et al*. (2020) found that germination was the point at which mungbean protein digestibility maximized. Other researchers have studied improvements in protein digestibility upon germination (Gu *et al.,* 2023).
The present investigation showed that the *in vitro* protein digestibility and *in vitro* starch digestibility of CF, GCF, and FCF were 70.41, 73.40, 80.13% and 36.33, 37.13, and 38.40%, respectively. *In vitro* protein digestibility increased during germination in GMF and GCF. Increases in the protein solubility of germinated legume seeds have been observed in recent studies (Bera *et al.,* 2023).

According to Gan *et al*. (2017) germination increases the quantity of phenolic compounds that interact with the proteins in the germinated legume seeds, increasing the proteins' digestibility. Protease inhibitors, phytic acid, polyphenols, and seed protein breakdown during germination may significantly increase the digestibility of proteins. According to Liu *et al*. (2024) there was a significant ($p \leq 0.05$) increase in the *in vitro* protein and starch digestibility after maize fermentation.

Water absorption capacity and swelling index

Water absorption capacity of WF, MF, GMF, FMF, CF, GCF, and FCF was observed at 147.00, 204.93, 239.20, 203.66, 196.10, 222.30, 202.03%, respectively (Fig 1). The higher value of water absorption capacity was observed in GMF (239.20 %), whereas the lowest value of water absorption capacity was observed in WF (147.00%). A significant difference ($P<0.05$) was observed in water observation capacity among all flours (Fig 1).

Water absorption capacity measures the amount of water used for gelatinization. It was found that the corn sample had a lower water absorption capacity than the other samples and that the GMF sample had a higher water absorption capacity. The control mungbean paste may have increased the mungbean flour's water binding capacity, which improved its textural properties and reconstitution ability. It may be related to the loss of starch polymer structure, as the lower value indicated the compactness of the structure (Dauda *et al.,* 2018). Another reason for a high-water absorption capacity is that starch polymers have a lost structure; a low value suggests that the structure is tightly packed. (Karaman and Turkay, 2023).

The swelling index of WF, MF, GCF, FMF, CF, GCF, and FCF was observed at 6.69, 5.21, 6.01, 3.95, 6.29,3.84 and 4.47, respectively (fig 2). Rizvi*et al*. (2024) found that the product's swelling capacity during germination impacts its other functional features,

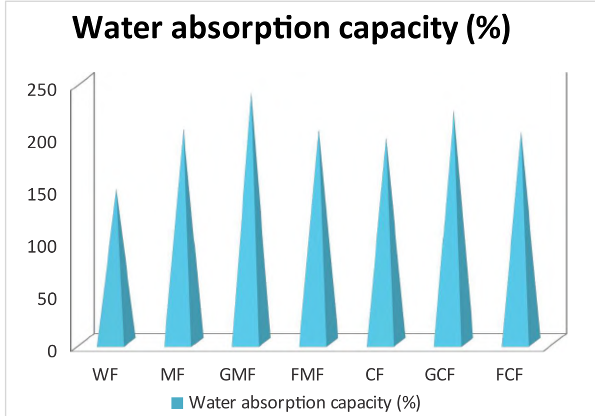

Fig.1: Water absorption capacity of Refined wheat flour (WF), mungbean flour (MF), germinated and fermented mungbean flour (GMF and FMF), corn flour (CF), and germinated and fermented corn flour (GCF and FCF). Values are expressed as mean ± standard deviation

Table 1: *In vitro* protein digestibility and *In vitro* starch digestibility

Flour	*In vitro* protein digestibility (%)	*In vitro* starch digestibility (mg malt/g)
WF	37.26±8.22[b]	35.33±3.05[abc]
MF	57.22±28.68[ab]	24.80±2.30[d]
GMF	63.02±26.32[ab]	27.73±3.008[cd]
FMF	68.91±8.54[a]	29.20±10.02[bcd]
CF	70.41±9.25[a]	36.33±2.54[ab]
GCF	73.40±13.69[a]	37.13±1.89[a]
FCF	80.13±7.45[a]	38.40±2.55[a]
C. D. at 5%	3.49	0.85

Values are mean ± SE of three independent determinations

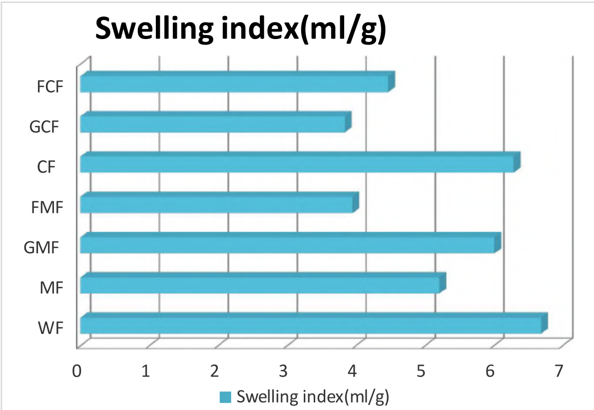

Fig.2: Swelling index of Refined wheat flour (WF), mungbean flour (MF), germinated and fermented mungbean flour (GMF and FMF), corn flour (CF), and germinated and fermented corn flour (GCF and FCF). Values are expressed as mean ± standard deviation

ultimately determining its eligibility for product development. According to Kaur *et al*. (2018) isolated cereal starches have a greater swelling capability, suggesting that cereal starches expand more readily than legumes. Flour may swell differently depending on the difference in starch granule structure between whole grain flour and grains and legumes, as well as pure starch versus whole grain flour. Germinated flour contained a lower swelling index because germination leads to the disruption of hydrogen atoms inherent in maize flour by amylases and proteases into sugars and amino acids, respectively.

Adejobi *et al*. (2024) observed a similar value of the swelling index of maize. According to Ola and Opaleye (2019), fermentation results show an increase in reconstitution index (4.82-6.40 ml / g) and a decrease in swelling index (6.47-3.46 ml / g) but an increase in water absorption capacity (3.70-3.92 g g^{-1}).

Effect of supplementation of RF, MF, GMF, FMF, CF, GCF and GCF on rheological properties

Pasting properties

A rapid viscoanalyzer was used to evaluate the pasting characteristics of refined wheat flour, MF, GMF, FMF, CF, GCF, and FCF. Table 2 shows peal 1, trough, breakdown, final viscosity, setback, peak

Table 2: Pasting properties of various composite flour

Flour	Peak 1 (cP)	Trough1 (cP)	Breakdown (cP)	Final viscosity (cP)	Set back (cP)	Peak time (Min.)	Pasting temperature (°C)	Gelatinization temperature (°C)
WF	3030 ± 62.23^{a}	2290 ± 139.08^{a}	806.66 ± 99.62^{b}	3704.66 ± 287.84^{a}	1148 ± 130.66^{a}	6.62 ± 0.08^{a}	66.63 ± 1.01^{c}	95.05 ± 0.01^{a}
MF	784.33 ± 20.64^{cd}	616 ± 101.05^{cd}	113.66 ± 8.32^{d}	1281 ± 91.53^{c}	531.66 ± 39.55^{b}	4.91 ± 0.44^{c}	80.55 ± 6.92^{a}	92.60 ± 2.29^{bc}
GMF	789 ± 72.74^{cd}	238.33 ± 16.86^{ef}	470.66 ± 150.14^{c}	317.00 ± 46.50^{d}	102.66 ± 12.89^{d}	4.71 ± 0.15^{cd}	81.85 ± 0.52^{a}	94.56 ± 1.59^{ab}
FMF	986.33 ± 69.02^{c}	762.66 ± 47.64^{bc}	223 ± 21.73^{d}	949.00 ± 57.71^{c}	186.33 ± 10.11^{cd}	5.43 ± 0.05^{b}	82.83 ± 0.59^{a}	95.11 ± 0.05^{ab}
CF	2162.67 ± 197.07^{b}	927.33 ± 44.85^{b}	1268.3 ± 150.59^{a}	2303 ± 84.00^{b}	1342.33 ± 56.51^{a}	4.40 ± 0.13^{d}	72.43 ± 0.41^{b}	91.20 ± 1.67^{c}
GCF	146.66 ± 79.10^{e}	132.22 ± 15.37^{f}	114.33 ± 70.61^{d}	743.66 ± 62.74^{c}	144.66 ± 29.73^{c}	3.86 ± 0.13^{e}	79.31 ± 0.17^{a}	84.83 ± 1.70^{d}
FCF	546.33 ± 53.57^{d}	304.00 ± 152.15^{cd}	130.33 ± 9.07^{d}	769.33 ± 64.70^{c}	353.33 ± 20.20^{bc}	5.49 ± 0.03^{b}	82.66 ± 0.46^{a}	95.13 ± 0.05^{a}
C. D. at 5%	1.41	4.98	6.13	5.47	9.08	0.33	0.68	2.43

Values are mean ± SE of three independent determinations.

time, pasting, and gelatinization temperature.

The results in Table 2 include peak 1, trough, breakdown, ultimate viscosity, setback, peak time, pasting, and gelatinization temperature. These characteristics are important for estimating the matrices' functional characteristics, how they will behave when subjected to heat and hydration limitations, how amyloidosis will retrograde, how much of their structure is amorphous, and how molecules will rearrange (Jia *et al.,* 2019; Rong *et al*.,2022; Xu *et al.,* 2023).

The results of the present experiments indicate that a non-significant difference was observed in GCF and FCF's breakdown, final viscosity, and pasting temperature. CF contained higher peak, trough, breakdown, final viscosity, and setback (2162.67, 927.33, 1268.3, 2303 and 1342.33) than MF, GMF, FMF, GCF, and FCF. The low degradation of germinated flour suggested that it had high thermal stability and high resilience to degradation. However, a lower setback after sprouting suggested a higher degree of product age and a stronger raw material processing relationship. Significant setbacks make the flour susceptible to ageing (Liu *et al.,* 2020). High setback viscosity reduces the retrogradation of the wheat paste during cooling and lowers the product's staling rate (Abegunde *et al.,* 2019). Peak viscosity indicates high starch content (Obinna-Echem*et al*., 2019). Treatments likefermentation and malting have been reported by Santos *et al*. (2024) to lower the percentageof retrogradation.

Several studies indicate that dough prepared using green gram-containing composite flours can have excellent rheological and functional characteristics for baked goods (Chandra *et al.,* 2015). According to research by Julianti *et al*. (2017) composite flours typically have lower rheological properties than wheat flour.

Quality analysis of value-added biscuits

Ten variety of biscuits *viz.,* sweet with W_0 (WF 100),C_1 (WF: MF: CF 25:37.5:37.5),C_2 (WF: MF: CF 50:25:25), C_3 (WF: MF:CF 75:12.5:12.5),G_4 (WF: GMF: GCF 50:25:25),G_5 (WF: GMF: GCF 50:25:25),G_6 (WF: GMF: GCF 75:12.5:12.5),F_7 (WF: FMF:FCF 25:37.5:37.5),F_8 (WF: FMF:FCF 50:25:25), F_9 (WF: FMF:FCF 75:12.5:12.5) respectively were evaluated for sensory analysis (Table 3).

Sensory analysis

All the biscuits were assessed for various sensory attributes using a 9-point hedonic scale. Table 3 describes the mean values of colour and appearance, taste, texture, aroma and flavour, after-taste, and overall acceptability.

Results showed that W_0, C_2, C_3, G_5, and F_9 can be incorporated into biscuit formulations without adversely affecting sensory attributes.

All the biscuits were assessed for various sensory attributes using a 9-point hedonic scale. Singh-

Table 3: Sensory analysis of biscuits prepared from different composite flours

Biscuits	Colour and appearance	Taste	Texture	Aroma and flavour	After taste	Overall acceptability
W_0	8.30±0.48^a	8.20±0.42^a	8.30±0.48^a	8.40±0.52^a	7.90±0.32^{ab}	8.22±0.30^a
C_1	7.90±0.32^a	8.00±0.47^a	8.20±0.63^a	8.10±0.74^a	7.70±0.48^{abc}	7.98±0.38^{ab}
C_2	8.29±0.48^a	7.90±0.74^a	8.11±0.57^{ab}	7.90±0.74^{ab}	7.90±0.57^{ab}	8.02±0.45^{ab}
C_3	8.30±0.48^a	8.00±0.74^a	8.10±0.57^{ab}	7.10±0.74^{ab}	7.90±0.57^{ab}	8.02±0.45^{ab}
G_4	7.90±0.57^a	7.90±0.74^a	7.70±0.48^b	7.70±0.82^b	8.10±0.57^a	7.86±0.45^{ab}
G_5	8.10±0.57^a	8.10±0.88^a	7.90±0.57^{ab}	8.00±0.67^{ab}	7.90±0.57^{ab}	8.00±0.42^{ab}
G_6	7.90±0.32^a	7.90±0.88^a	7.70±0.48^b	7.90±0.74^{ab}	7.70±0.48^{abc}	7.82±0.36^b
F_7	7.00±0.47^b	6.70±0.67^b	6.70±0.67^c	6.80±0.42^c	7.50±0.53^{bcd}	6.94±0.38^c
F_8	7.10±0.57^b	6.90±0.74^b	6.90±0.57^c	6.90±0.32^c	7.30±0.48^{cd}	7.02±0.38^c
F_9	7.30±0.82^b	7.10±0.99^b	6.80±0.63^c	6.90±0.74^c	7.10±0.57^d	7.04±0.60^c
C. D. at 5%	0.46	0.66	0.50	0.58	0.46	0.37

Values are mean ± SE of ten independent determinations.

Ackbarali and Maharaj (2014) used a 9-point hedonic scale to assess the sensory qualities of appearance, taste, texture, aroma, and overall acceptability (1- dislike extremely, 5- neither like nor dislike, 9- like extremely). Ten semi trained panellists analyzed the biscuits' sensory analysis (Agrahar-Murugkar and Jha, 2011). The results of the present experiments, the mean value of colour and appearance, taste, texture, aroma and flavour, after-taste, and overall acceptability are described in Table 3.

Mean values of W_0 colour and appearance, taste, texture, aroma and flavour, after taste, and overall acceptability were 8.30, 8.20, 8.30, 8.40, 7.90, and 8.22, respectively. The Maillard reaction occurs when sugar reacts with a restricted amino group found in proteins or amino acids, causing mungbean biscuits to become darker in colour the longer they bake. The characteristic aroma and brown colour are produced by the Maillard process (Starowicz and Zielinski, 2019). According to Setyaningsih *et al*. (2019) baking at high temperatures for extended periods will result in a darker-coloured biscuit having less water. According to Lean (2013), the biscuit's taste, colour, and aroma had been modified as an effect of baking. C_1, C_2& C_3 biscuits contained mean values of colour and appearance (7.90, 8.29 and 8.30), taste (8.00, 7.90 and 8.00), texture (8.20, 8.11 and 8.10), aroma and flavour (8.10, 7.90 and 7.10), after taste (7.70, 7.90 and 7.90) & overall acceptability (7.98, 8.02 and 8.02) respectively. Regarding overall acceptability scores, C_2 and C_3 biscuits contained 'liked moderately' to 'liked very much' among control biscuits. Similar scores for all sensory attributes were observed by Canali *et al*. (2020). According to Sangwan and Dahiya's (2013) research, biscuits made with composite flour in various ratios were "liked moderately" based on sensory evaluation.

Results of the present experiments indicate that a significant difference ($P<0.05$) was observed between G_4, G5, and G_6 biscuits in all sensory attributes except colour appearance and taste (Table 4.10). G_4, G_5& G_6 biscuits contained mean score of colour and appearance were (7.90, 8.10 and 7.90); taste was (7.90, 8.10 and 7.90); texture was (7.70, 7.90 and 7.70); aroma and flavour were (7.70, 8.00 and 7.90); after taste was (8.10,7.90 and 7.70) & overall acceptability was (7.86, 8.00 and 7.82) respectively. As a result, the G_5 biscuit was adjudged 'liked very much' by sensory panel members. According to Jiao *et al*. (2016) the interaction of sugar, margarine, and baking powder with the evaporation of volatile chemicals produces the aroma of biscuits.

The mean values of F_7, F_8 & F_9 biscuits were 7.00, 7.10 and 7.30 in colour and appearance,6.70, 6.90 and 6.80 taste, 6.70, 6.90 and 6.80 in texture,6.80, 6.90 and 6.90 aroma and flavour,7.50,7.30 and 7.10 after taste & 6.94,7.02 and 7.04 overall acceptability respectively. Results indicate that F_9 biscuits were adjudged' liked moderately' and possessed maximum mean scores of all sensory attributes compared to F7 and F_8 biscuits. The samples of fermented biscuits had texture scores that resembled those of our earlier research, which showed that fermented biscuits had higher textural characteristics (Adebiyi*et al*., 2017). Biscuits containing sprouted gram and mung flour had higher colour, crunchiness, and overall acceptability scores than the control (Gupta and Bhatt, 2023).

CONCLUSION

Refined wheat flour is commonly used for bakery products and is nutritionally inferior. Cereal and legumes could be added along with wheat flour to make bakery products nutritive. Corn and mung beans, abundant in essential nutrients, are prime selections for these processes. Further, the nutritive value of bakery products can be enhanced by germination and fermentation of cereal and pulse. This improves protein, dietary fibre, and rheological and sensory properties. It is also helpful in increasing in vitro starch and protein digestibility.

In conclusion, incorporating germinated and fermented mung bean and corn flours into bakery products positively affects their functional and rheological properties, improving sensory attributes. These findings underscore the potential of pre-digested composite flours as a valuable ingredient for creating bakery products that are not only nutritious but also functionally superior and appealing to consumers.

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