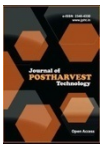

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RESEARCH ARTICLE

Impact of sprouted gram and mung flour on iron, calcium, dietary fiber, and vitamin C content in biscuits

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ABSTRACT

This study aimed to investigate the potential impact of sprouted gram and mung flour on the nutritional content of biscuits. Six variations of biscuits were prepared, each with different ratios of refined flour, sprouted gram flour, and sprouted mung flour. All samples contained 1 gram of baking powder, 1 gram of baking soda, 60 grams of sugar, 50 grams of butter, and sufficient water to make a soft dough. The results of the study showed that the incorporation of sprouted gram and mung flour into biscuits led to an increase in Calcium and Vitamin C content. Sample T3, which contained 70% refined flour, 15% sprouted gram flour, and 15% sprouted mung flour, had the highest levels of Calcium (260 ± 25.1 mg/100g) and Vitamin C (26.1 ± 0.406 mg/100g). The analysis conducted to determine the iron content in all six samples was unsuccessful as the results were below the limit of quantification (BLQ), implying that the iron concentration was too low to be accurately measured using the employed method. In conclusion, the incorporation of sprouted gram and mung flour into biscuits can increase their nutritional value. Therefore, these flours can be considered as an alternative ingredient in the production of biscuits. Further studies could explore the potential impact of sprouted gram and mung flour on other nutritional components of baked goods.

Keywords: Sprouted gram, sprouted mung, value addition, biscuit, fortified food

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INTRODUCTION

Egypt, the Ministry of Agriculture, has recently introduced mung bean (*Phaseolus aureus*) as a new crop. Mung bean is a highly nutritious food, containing 27% protein. The essential amino acid composition of mung bean is comparable to that of soybean, kidney bean, and the reference protein established by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). This makes mung bean an excellent source of protein that can contribute to a healthy and balanced diet (El-Adawy et al., 1996).

Chickpeas (*Cicer arietinum* L.) are a legume that has been consumed widely for centuries, particularly in tropical and subtropical regions. The Kabuli variety of chickpea seeds is primarily grown in the Mediterranean, Near East, Central Asia, and America, as

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documented by (Sing et al., 1991). These seeds are notable for their large size and salmon-white color, as well as their high levels of carbohydrate (41.1-47.4%) and protein (21.7-23.4%). Starch constitutes the majority of the carbohydrate fraction, accounting for approximately 83.9% of total carbohydrates, as reported by (Rincón et al., 1998).

Biscuits are a popular ready-to-eat snack due to their wide consumption base, availability, longer shelf-life, affordability, diverse flavours, and good quality, as documented in various studies including (Singh et al., 1993; Gandhi et al., 2001; Hooda and Jood et al., 2005). To improve the nutritional value of biscuits, several research studies have been conducted using legumes like chickpea, pigeon pea, moong bean, cowpea, and green lentils (Thongram et al., 2016). These legumes have been added to wheat flour to create high protein and high fiber biscuits. Some examples of these studies include (Tiwari et al., 2011; Zucco et al., 2011).

Biscuits are a popular snack food consumed worldwide, with various types and flavours available in the market. However, most commercial biscuits are made using refined flour, which is low in nutritional value and may contribute to health problems such as obesity and diabetes (Gaesser et al., 2019) As a result, there is a growing demand for healthier snack options that can provide both taste and nutrition.

Vidal-Valverde et al. (2002) suggested that germination is a cost-effective method that can significantly modify the nutritional composition of beans, lentils, and peas. The nutritive value of the germinated product depends on the germination conditions. Germination is an effective approach to reduce flatulence caused by lentils, while also improving the digestibility of starch despite lower starch content. Moreover, germination leads to an increase in crucial dietary fiber components like cellulose and lignin, which hold significant physiological importance (Vidal-Valverde et al., 1992). The germination process has been found to have a significant impact on the nutritional and sensory properties of soybean seeds. Ahmad et al. (2000) reported a significant increase in ascorbic acid and riboflavin content and a notable change in thiamine content after germination. However, there is limited research on the use of sprouted green gram and sprouted gram flour in biscuits. Therefore, the aim of this study was to develop and evaluate the sensory and textural characteristics of sprouted green gram and sprouted gram flour biscuits, and compare them to traditional biscuits made with refined flour. This study builds upon previous research and extends the knowledge on the potential use of sprouted green gram and sprouted gram flour in baked goods.

MATERIALS AND METHODS

The present study was conducted at the Department of Food Technology, Bundelkhand University, Jhansi (Uttar Pradesh), India. Good quality Green Gram (Mung Bean or Moong) with the botanical name *Vigna radiata* L. *Wildzek* and Gram (Chickpea or Chick Pea or Bengal Gram) with the botanical name *Cicer arietinum* were purchased from the local market. The refined flour, sugar, butter, baking powder, and baking soda used in the study were also purchased from the local market in Jhansi, UP.

Blending ratio

The blending ratio of the three flours (Table 1) was varied in each sample of biscuits.

- For the control sample (T0), 100% refined flour was used.
- In sample T1, 90% refined flour, 5% sprouted gram flour, and 5% sprouted mung flour were used.
- In sample T2, 80% refined flour, 10% sprouted gram flour, and 10% sprouted mung flour were used.

- In sample T3, 70% refined flour, 15% sprouted gram flour, and 15% sprouted mung flour were used.
- In sample T4, 60% refined flour, 20% sprouted gram flour, and 20% sprouted mung
- flour were used.
- In sample T5, 50% refined flour, 25% sprouted gram flour, and 25% sprouted mung flour were used. Explain in table 1

Table 1: Blending ratio of different flours in each sample of biscuits

Sample	Refined Flour (%)	Sprouted gram Flour (%)	Sprouted mung Flour (%)
T0	100	0	0
T1	90	5	5
T2	80	10	10
T3	70	15	15
T4	60	20	20
T5	50	25	25

These variations in the blending ratio of the flours allowed for the evaluation of the impact of sprouted gram flour and sprouted mung flour on the nutritional value, physical properties, and sensory attributes of the biscuits.

Preparation of sprouted flour

Cleaned seeds of both gram were soaked in water for 8 to 10 hours at room temperature. The unabsorbed water was discarded and soaked seeds were rinsed twice with potable water to avoid post-contamination during germination. The soaked seeds were allowed to germinate for 72 hours at room temperature, with frequent watering. The germinated green gram and gram seeds were dried in a cabinet tray dryer at 50-60°C for 5 to 6 hours and the ground into fine powder. The flour was passed through a sieve to obtain a smooth texture by Yasmin et al. (2008) with some modification. For each sample of biscuits, 1 gram of baking powder and 1 gram of baking soda were used. The water was added in sufficient quantity to make a soft dough, and 60 grams of sugar and 50 grams of butter were used for each sample.

Preparation of biscuits

The biscuits were formulated according to the recipe given by AACC (2000) with slight modifications. Fat and sugar were mixed until light and fluffy. Then, composite flour sprouted green gram flour, sprouted gram flour and refined flour and other ingredients such as baking powder and baking soda were added and mixed thoroughly. Water was added to form a soft dough, which was rolled out into sheets and cut into circles. The biscuits were baked at 200°C for 20 minutes (until proper colour developed), cooled, and stored in a high-density polyethylene jar at room temperature for further analysis (Fig. 1).

Analytical methods

The nutritional composition of the six biscuit samples was analysed using standard analytical methods. The iron, vitamin, dietary fiber, and calcium content of the sprouted gram and mung flour biscuits were determined through tests conducted at the Delhi Analytical Research Laboratory.

Calcium

The determination of calcium involves taking a suitable amount of the sample solution containing approximately 5 to 20 mg of calcium as CaO and adding triethanolamine, hydroxylamine hydrochloride, and potassium cyanide solutions to a 500 ml conical flask. The solution is then diluted, and the pH is adjusted to 12.5-13 using a pH indicator paper or meter. Patton and Reeder's indicator mixture is added, and the solution is titrated with 0.01 M EDTA solution until the color changes from red to pure blue free from any violet tinge. The calcium (as Ca) percent by mass is calculated using the formula: Calcium percent by mass = (V1 x 0.04008) / M, where V1 is the volume in ml of EDTA solution consumed in titration, and M is the mass in grams of the sample in the solution taken for the test. It is important to note that if the solution contains ammonium salts, the amount of sodium hydroxide solution required to adjust the pH may be higher, and the pH must be brought to 12.5-13 to ensure accurate results (Indian Standard: Bureau of Indian Standards, 1967).

Dietary fiber

The sample residue is subjected to pepsin digestion, followed by pancreatin and glucoamylase digestion. The resulting insoluble fraction is washed with acetone and ether and dried, while the soluble fraction is washed with ethyl alcohol, acetone, and ether and dried. The total dietary fiber is calculated by adding the mass of the soluble and insoluble fractions and expressing it as a percentage of the sample mass on a dry mass basis, after correcting for the lipid content (Indian Standard: Bureau of Indian Standards, 1984).

Total Dietary fiber = ((Mass of soluble fraction + Mass of insoluble fraction) / Mass of the sample) x 100

Vitamin C and iron contents

Five grams of the sample was ground in a mortar with acid washed sand using TCA reagent or metaphosphoric acid. It was then transferred into a 100-ml graduated flask and stirred thoroughly. Make up the volume to 100 ml with TCA reagent or metaphosphoric acid. Filter the mixture immediately through a fluted filter paper. The final concentration of ascorbic acid in the extract should be 10 to 15 mg per ml and titrated against the indophenol solution. The iron content was estimated using a standard method.

Data analysis

The study investigated the levels of calcium, dietary fiber, vitamin C, and iron in six different samples of biscuits, each with varying proportions of refined flour, sprouted gram flour, and sprouted mung flour (labelled T0-T5). The mean values and standard deviations for each nutrient were calculated from three replicates. Statistical analysis was performed to determine whether there were significant differences between the samples.

The statistical analysis was conducted using the R programming language and the Tukey's test was applied with a significance level set at 5%. The Tukey's test determines whether there are significant differences between means by calculating the critical difference (C.D.), which represents the minimum difference between means that is required to be considered statistically significant at a 95% confidence level.

In case there are multiple variables being measured in the study, an ANOVA (Analysis of Variance) can be used to determine if there are any significant differences among the groups. The ANOVA would require calculating the means and standard deviations for each group for each variable and then comparing them using an F-value and p-value.

RESULTS AND DISCUSSION

The analysis results showed significant differences in all three nutritional components (calcium, dietary fiber, and vitamin C) among the samples (T0-T5). The p-values (<0.05) and F-values indicated that there were significant differences between the samples (Table 2). Sample T3, which contained 70% refined flour, 15% sprouted gram flour, and 15% sprouted mung flour, had the highest levels of calcium and vitamin C. It also had the highest mean square value and F-value among all the samples, indicating a significant difference from the other samples. Samples T3 and T4 had significantly higher levels of calcium than the other samples. Sample T1 had significantly lower levels of vitamin C compared to the other samples, while samples T3 and T4 had significantly higher levels of vitamin C. Finally, sample T1 had significantly lower levels of dietary fiber compared to the other samples, while sample T4 had significantly higher levels of dietary fiber.

Table 2: Values are mean of three replicates ± SD, means with different letters at the same column are significantly different at P ≤ 0.05 (*p<0.05, **p<0.01, ***p<0.001)

Sample	Calcium	Dietary Fiber	Vitamin C
T0	147 ± 1.57 ^{cd}	4.29 ± 0.07 ^{a}	21.1 ± 0.079 ^{c}
T1	150 ± 2.3 ^{cd}	4.31 ± 0.035 ^{a}	14.8 ± 0.129 ^{e}
T2	191 ± 0.526 ^{b}	4.31 ± 0.045 ^{a}	19.5 ± 0.235 ^{d}
T3	260 ± 25.1 ^{a}	4.28 ± 0.065 ^{a}	26.1 ± 0.406 ^{a}
T4	134 ± 0.255 ^{d}	4.42 ± 0.06 ^{a}	21.5 ± 0.065 ^{c}
T5	173 ± 0.905 ^{bc}	4.4 ± 0.051 ^{a}	23.3 ± 0.182 ^{b}
Mean Square	6283.7	0.01065	43.512
F value	58.873	3.4171	930.3
p-value	5.031e-08 ***	0.03767 *	4.242e-15 ***
C.D.	30.7797	0.166326	0.644331

However, the Vitamin C content in samples T1-T5 was significantly lower than that of the control sample (T0), which used only refined flour. Dietary fiber content did not differ significantly among the samples. It is important to note that the intended measurement of iron content in all six samples was not successful, as the analysis yielded results below the limit of quantification (BLQ), indicating that the concentration of iron was too low to be accurately measured by the method utilized in the analysis.

Sample T5 had a calcium content of 173 ± 0.905 mg/100g, which is higher than the calcium content of samples T1, T2, and T6. However, the calcium content of T3 and T4 was even higher than that of T5, with T3 having the highest calcium content among all the samples. The letters (a, b, c, etc.) after the mean values in the table indicate significant differences between the samples for a particular nutrient. Samples that share the same letter are not significantly different from each other, while samples with

different letters are significantly different from each other at the 5% level of significance. In this case, sample T5 has the letter "b" after its mean calcium value, indicating that it is not significantly different from samples T3 and T4 (which have the letter "a"), but it is significantly different from samples T1, T2, and T6 (which have the letter "c").

Overall, the results suggest that the addition of sprouted gram and mung flour to biscuits can significantly increase their calcium and vitamin C content. However, the vitamin C content is still lower than that of biscuits made with only refined flour. Future studies may investigate the potential of other sprouted flours and ingredients to further enhance the nutritional profile of biscuits.

Prathusha and Cynthia (2022) reported that the sprouted green gram incorporated cookies exhibited a considerable iron content of 0.42 mg/100g. This finding is noteworthy as it indicates the potential of incorporating sprouted green gram as a source of dietary iron in cookie formulations. The iron content in the cookies could contribute to meeting the recommended daily intake of iron and help address iron deficiency among consumers.

Mubarak et al. (2005) found that boiling, autoclaving, and microwave cooking had minimal effects on the crude fiber content of mung bean seeds. The crude fiber content of the processed samples ranged from 4.50% to 4.64%, indicating that these cooking methods had little impact on the fiber content. This suggests that the fiber content remained relatively stable during these cooking processes. The effect of traditional domestic processes on the calcium content of mung bean seeds (mg/100 g dry weight basis) was investigated (Mubarak et al., 2005)). The results showed that boiling, autoclaving, and microwave cooking led to a slight decrease in calcium content compared to the raw samples. The calcium content ranged from 75.00 mg/100 g to 88.50 mg/100 g in the processed samples, with the highest value observed after germination. However, these differences were not statistically significant, indicating that the cooking methods had minimal impact on the calcium content of mung bean seeds.

The effect of different cooking methods on the crude fiber content of chickpea seeds (g/100 g-dry weight basis) was studied by El-Adawy (2002). The results showed that boiling, autoclaving, microwave cooking, and germination did not significantly affect the crude fiber content compared to the raw samples. The crude fiber content ranged from 3.82 g/100 g to 5.21 g/100 g in the processed samples, with no significant differences observed among the treatments. This suggests that the cooking methods and germination had minimal impact on the crude fiber content of chickpea seeds. In the study conducted by El-Adawy (2002), it was reported that the calcium content of chickpeas (*Cicer arietinum* L.) varied among different cooking methods and germination. The results showed that the calcium content in the raw chickpeas was 176 mg/100g. Among the processed samples, including boiling, autoclaving, microwave cooking, and germination, the calcium content ranged from 124 mg/100g to 131 mg/100g. Germinated chickpeas exhibited the highest calcium content compared to the other cooking methods, with a calcium content of 506 mg/100g.

Yousaf et al. (2013) in their study analysed the proximate composition of the gram flour supplemented cookies. The treatments included different percentages of gram flour (ranging from 0% to 50%) in the cookies. Calcium (Ca) Content: The results showed that as the percentage of gram flour increased in the cookies, the calcium content also increased. The highest calcium content was observed in the treatment group with 50%-gram flour (T5), while the control group (T0) had comparatively lower calcium levels. Fiber Content: Similarly, an increase in the percentage of gram flour resulted in higher fiber content in the cookies. The treatment group with 50%-gram flour (T5) had the highest fiber content, while the control group (T0) had relatively lower fiber levels.

CONCLUSION

In conclusion, this study showed that incorporating sprouted gram and mung flour into biscuit production can significantly enhanced the calcium and vitamin C content of the product. Sample T3, which contained 15% sprouted gram flour and 15% sprouted mung flour, had the highest levels of calcium and vitamin C among all the samples tested. However, there was no significant difference in the dietary fiber content between the different types of biscuits. Concentration of iron was too low to be accurately measured by the method utilized in the analysis in all sample. The use of sprouted flour in biscuits can provide an alternative market for sprouted grains, which are often underutilized in the food industry. These findings suggest that sprouted flours can be used as a functional ingredient in bakery products to improve their nutritional value without compromising their sensory quality. Further studies can be conducted to explore the potential of sprouted flours in other baked goods and investigate the potential health benefits of consuming sprouted flour-based products.

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