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# Investigating the Effect of Sprouting and Fermentation Processes to Improve the Nutritional Value of Mung Bean

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**ABSTRACT**

Sprouted legumes have recently received a lot of interest in the food business. In this work, the impacts of the germination process and the combined germination and fermentation with Saccharomyces cerevisiae on the physicochemical, qualitative, and nutritional aspects of mung bean were investigated. Moisture, protein, fat, ash, starch, sugar, phytic acid, total phenolics, antioxidant activity, water-soluble vitamins, and amino acid profiles were assessed in various mung bean samples. The results revealed that the fermentation and germination processes reduced moisture (6.85%) and starch (29.84%) while increasing sugar (2.2 mg/mL), fat (2.38%), and ash (5.27%). Due to the release of bound phenolics during germination and their higher bioavailability during fermentation, total phenolic compounds (72.01 mg gallic acid per gram of dry weight) and DPPH radical-scavenging antioxidant activity (74.32%) increased with fermentation. Degrading enzymes during fermentation and germination caused the amount of phytic acid to decrease to 0.64 mg per 100g. As the fermentation and germination processes progressed, the concentrations of water-soluble vitamins rose. Yeast activity is responsible for the increase in protein content (29.83%) and amino acids that resulted from germination and fermentation. The utilization of germination and fermentation procedures to increase the nutritional content of mung beans is positively evaluated by the study's findings.

## 1- Introduction

Legumes are considered a rich source of protein; however, their protein quality is inferior to that of meat due to the deficiency of sulfur-containing amino acids such as methionine and cysteine. Additionally, legumes contain various antinutritional factors such as protease inhibitors, α-amylase inhibitors, tannins, phytic acid, alkaloids, and glycosides, which reduce their overall nutritional quality [1]. One of the main advantages of mung beans is that they do not require nitrogen fertilization. Although mung beans are recognized as a rich source of dietary fiber, minerals, and proteins, they are also considered a source of various bioactive compounds. These compounds have been shown to exhibit physiologically beneficial effects such as antioxidant, antidiabetic, and anticancer properties, aiding in the prevention and control of various diseases. Mung beans are therefore regarded as a valuable protein source [2].

During the germination process, complex physicochemical and biochemical reactions occur, leading to significant changes in the composition and morphology of legumes. These processes involve the breakdown of seed storage compounds and the synthesis of structural proteins. In recent years, germination of cereal-based products has been widely employed to develop new products with enhanced nutritional value and improved nutrient bioavailability [3]. Products derived from germinated raw materials are reported to have a softer texture and a noticeably sweeter taste. The growing consumer demand for whole grain foods and high-quality products has led to the development of innovative technologies aimed at creating naturally flavored foods, with reduced use of chemical flavor and texture enhancers, through methods such as germination and fermentation [4].

Germination has a significant impact on the nutritional quality of cereals. It leads to alterations in the quantity and type of nutrients within the seed, which can vary depending on the plant species, seed type, and germination conditions [5]. During germination, the availability of minerals and vitamins increases, alongside enhanced antioxidant activity and a reduction in antinutritional factors such as phytates. Germination is a natural biological process in plants that transitions seeds from dormancy to active growth [6]. Both germination and fermentation processes activate endogenous enzymes, thereby improving the digestibility of the seeds. Furthermore, these processes increase the availability of reducing sugars and free amino acids, including lysine. Food processing treatments such as fermentation, germination, cooking, soaking, and roasting affect both the nutritional composition and physicochemical characteristics of the final product [7]. Therefore, the aim of this study is to investigate the effects of germination and fermentation on mung beans to enhance the nutritional value of food matrices developed from them.

## 2-Materials and Methods

### Materials

The raw material used in this study was a local variety of mung bean (Vigna radiata) obtained from the local market of Isfahan, Iran. All chemical reagents were purchased from Merck (Germany) and Dr. Mojallali Chemical Company (Iran).

## Methods

### Preparation of Mung Bean Samples

First, the mung beans were thoroughly cleaned to remove any impurities and foreign matter. They were washed with tap water and then disinfected using a 7% sodium hypochlorite solution for 30 minutes. After disinfection, the mung beans were divided into two portions. One portion was dried in an oven without undergoing germination. The second portion was soaked in distilled water for 24 hours at 25 ± 2 °C. After soaking, the seeds were allowed to germinate at 25 °C for 72 hours. Germinated seeds with more than 90% uniform sprouting were selected and dried in an oven at 65 °C for 24 hours. The dried sprouts were milled using a laboratory grinder equipped with a 0.7 mm mesh sieve and stored in zip-lock plastic bags at 4 °C until further analysis.

To prepare fermented germinated mung beans, approximately 7 grams of Saccharomyces cerevisiae was dissolved in 65 mL of water. After a 5-minute activation period, the yeast solution was added to the germinated mung beans and allowed to ferment for 12 hours. The fermented samples were then dried in an oven at 65 °C, milled using a 0.7 mm mesh sieve, and stored in zip-lock plastic bags at 4 °C until analysis.

### Mung Bean Analysis

The following parameters were measured in mung bean samples: moisture content [8], fat content [9], ash content [8], protein content [10], soluble sugars and starch [11], phytic acid [12], total phenolic content, and antioxidant activity [13]. Water-soluble vitamins [14] and amino acid profiles [15] were analyzed using High-Performance Liquid Chromatography (HPLC) with an Agilent 1260 Infinity LC system equipped with an Agilent EC-C18 column.

### Statistical Analysis

A completely randomized design (CRD) was employed in this study. The independent variables included the control sample, germinated sample, and germinated-fermented sample, each at a single level. Dependent variables included physical and chemical properties of mung beans. Statistical analyses were performed using SPSS software, and means were compared using Duncan's multiple range test at a 5% significance level ($p < 0.05$). All graphs were generated using Microsoft Excel.

## Results and Discussion

### Effect of Germination and Fermentation on the Proximate Composition of Mung Beans

The results of the proximate composition analysis of mung bean samples subjected to germination and fermentation are presented in Table 1. The lowest moisture content (6.85%) was observed in the germinated and fermented sample, while the highest moisture content (10.35%) was recorded in the germinated sample. Germination led to an increase in moisture content due to water absorption necessary for initiating metabolic activity. However, fermentation following germination resulted in a reduction in moisture content.

Talebi Najafabadi et al. (2019) similarly reported that moisture content increased with longer germination time, consistent with the present study [16]. As germination progresses, the seed absorbs more water to support biological activity, explaining the observed moisture increase [17]. These findings are in agreement with Enaemene et al. (2020), who noted reduced moisture content in germinated and fermented maize compared to control samples [14]. The increased number of cells during sprouting further contributes to water uptake and hence higher moisture levels post-germination [18].

Other studies, including Megat-Rusydi et al. (2011), Khare et al. (2021), and Enaemene et al. (2022), have reported similar moisture increases after germination in mung beans, peanuts, soybeans, rice, flaxseed, and chickpeas [8, 19, 20]. Wakil and Kazim (2012) investigated the effects of starter and controlled fermentation on a sorghum-bean composite, reporting a decrease in moisture content [21].

## Ash Content

Ash content results for various mung bean samples are shown in Table 1. Germination led to a statistically significant reduction in ash content. However, fermentation following germination increased ash content, with the highest value (5.27%) observed in the fermented sample.

Since ash represents the mineral content of food, its level generally correlates with the overall mineral composition. The decrease in ash content after germination may result from increased enzymatic activity, nutrient translocation to sprouts, leaching of minerals during soaking, and cellular division—all contributing to mineral redistribution and partial loss [23–25].

Conversely, fermentation with Saccharomyces cerevisiae led to higher ash content. During fermentation, organic compounds, especially carbohydrates, may be broken down or consumed, increasing the relative proportion of minerals and resulting in higher ash content. Cell wall modifications during fermentation may also release bound minerals. Moreover, S. cerevisiae can secrete enzymes that degrade organic matter, improving mineral bioavailability. Fermentation and germination also reduce anti-nutritional factors like phytates that bind minerals, further increasing measurable mineral content [23–25].

These findings are in line with those of Enaemene et al. (2022), Aikoghenelola and Adurotoye (2014), and others, who reported increased ash content in chickpeas, cowpeas, and other legumes post-germination and fermentation [8, 22, 26–28].

## Protein Content

Protein content results (Table 1) show that the control sample had the lowest protein content (27.52%), while the germinated and fermented sample exhibited the highest (28.83%). Both germination and fermentation contributed to increased protein levels.

During germination and fermentation, carbohydrates and fats are metabolized as energy sources, reducing their proportions and relatively increasing protein concentration. Moreover, germination triggers the synthesis of new proteins—primarily enzymes and structural proteins—to support seedling development. Fermentation with S. cerevisiae also contributes protein via microbial biomass. Microbial degradation of anti-nutritional factors like phytates further enhances protein availability and digestibility [29–31].

Nakata et al. (2018) noted that apparent protein increase may result from dry matter loss due to carbohydrate and fat hydrolysis [29]. Similar increases in protein content due to fermentation were reported by Oyarekua (2013), Wakil and Kazim (2012), and others, depending on fermentation parameters such as pH, temperature, and fermentation method (co- or mono-fermentation) [21, 32, 33].

## Fat Content

Table 1 shows that germination slightly reduced fat content, though not significantly. In contrast, fermentation after germination significantly increased fat content. During germination, stored lipids serve as energy sources, undergoing oxidation, which decreases measurable fat levels [37, 38].

Fermentation, however, particularly with S. cerevisiae, may increase fat content by synthesizing microbial lipids or liberating previously bound lipids. These changes may result in a measurable increase in fat content in fermented samples. Similar trends have been reported in millet and quinoa by Mbaye-Ndoye & Ibta (2016) and Pilco-Quesada et al. (2020), respectively [35, 39].

**Table 1.** Analysis of different compositions of mung beans

| Treatment | Moisture (%) | Ash (%) | Protein (%) | Carbohydrate (mg/ml) | Starch (%) | Fat (%) |
|---|---|---|---|---|---|---|
| RMB | 8.37$^{b}$ | 4.69$^{b}$ | 27.52$^{c}$ | 2.1$^{a}$ | 34.39$^{a}$ | 1.80$^{b}$ |
| SMB | 10.35$^{a}$ | 3.04$^{c}$ | 28.75$^{b}$ | 2.3$^{a}$ | 31.39$^{b}$ | 1.59$^{b}$ |
| SFMB | 6.85$^{c}$ | 5.27$^{a}$ | 29.83$^{a}$ | 2.2$^{a}$ | 29.84$^{c}$ | 2.38$^{a}$ |

Different letters in each column indicate statistically significant differences at the 5% level. . RMB: Raw mung bean sample, SMB: Sprouted mung bean sample, SFMB: Sprouted and fermented mung bean sample.

## Simple and Complex Carbohydrates

Changes in simple sugars and complex carbohydrates (starch) are shown in Table 1. Germination led to a non-significant increase in sugars ($p > 0.05$) and a significant reduction in starch ($p < 0.05$). Fermentation post-germination significantly increased sugar and decreased starch levels.

Hydrolytic enzymes such as amylases become active during germination, breaking down starch into simpler sugars like glucose and maltose [40]. Fermentation further enhances this process. Yeast initially increases sugar levels by breaking down complex carbohydrates, although sugars are later consumed during fermentation. These findings are in agreement with Verma et al. (2021), Sujka et al. (2013), and Qin Ma et al. (2015), who observed similar reductions in starch and increased sugar levels during germination and fermentation of legumes and grains [43–45].

## Phenolic Compounds and Antioxidant Activity

Table 2 presents data on total phenolic content and DPPH radical scavenging activity. Germination and fermentation both significantly increased phenolic content and antioxidant activity. A strong positive correlation was observed between phenolic content and antioxidant capacity.

Enzymes such as β-glucosidase, activated during germination, break down the seed cell wall, releasing bound phenolics [46]. During fermentation, phenolic compounds—such as phenolic acids and flavonoids—undergo biotransformation, increasing their bioavailability and antioxidant potential [47].

Melini & Melini (2021) demonstrated that fermentation enhances antioxidant activity in quinoa via phenolic release. Fermentation can convert bound phenolics into free forms, improving absorption and efficacy [48]. Similar results have been reported for green peas, brown rice, and lentils [49–52].

## Phytic Acid Content

Phytic acid levels in mung bean samples are reported in Table 2. Both germination and fermentation significantly reduced phytic acid content. Germination activates endogenous phytase enzymes, which break down phytic acid into simpler compounds, improving mineral availability. Fermentation further enhances this degradation by microbial phytase activity [53].

**Table 2.** Total phenolic content (TPC), antioxidant activity (AA), acid phytic content, and vitamines of different mung beans

| Treatment | TPC (mg Gallic acid/g dry weight) | AA (% of DPPH radical inhibition) | Phytic acid (mg/100g dry weight) | Vitamin (mg/kg dry weight) | | | |
|---|---|---|---|---|---|---|---|
| | | | | C | $B_{12}$ | $B_3$ | $B_1$ |
| RMB | 34.80$^c$ | 38.17$^c$ | 1.44$^a$ | 100$^b$ | 166.7$^b$ | 19.8$^b$ | 167.6$^c$ |
| SMB | 50.72$^b$ | 50.92$^b$ | 1.16$^b$ | 161$^a$ | 170.9$^b$ | 12.8$^c$ | 306.5$^a$ |
| SFMB | 72.01$^a$ | 74.32$^a$ | 0.64$^c$ | 102$^b$ | 251.9$^a$ | 21.3$^a$ | 202.6$^b$ |

Different letters in each column indicate statistically significant differences at the 5% level. RMB: Raw mung bean sample, SMB: Sprouted mung bean sample, SFMB: Sprouted and fermented mung bean sample.

The synergistic effect of combined germination and fermentation significantly lowers phytic acid content, aligning with findings from Arshad et al. (2023), Rico et al. (2021), and Enaemene et al. (2022) [8, 54–56].

## Water-Soluble Vitamins

Table 2 also summarizes the effects of germination and fermentation on water-soluble vitamins. Germination increased vitamins B1, B12, and C, while vitamin B3 decreased. However, the combination of germination and fermentation significantly enhanced all measured vitamins.

Germination boosts vitamin B1 and C through enzymatic synthesis. Fermentation, especially with S. cerevisiae, contributes additional B vitamins—most notably B12, which is not produced in plants but can be synthesized by certain microbes. These results are consistent with those of Pinheiro et al. (2021) and other researchers [15, 42].

## Amino Acid Profile

Table 3 presents the amino acid profiles of the mung bean samples. The control group had the lowest levels of most amino acids, while the germinated and fermented sample exhibited the highest. Both germination and fermentation improved protein quality and the bioavailability of essential amino acids such as lysine, methionine, and tryptophan.

Germination promotes enzymatic hydrolysis of storage proteins into free amino acids and reduces anti-nutritional factors, improving amino acid availability. S. cerevisiae also contributes by synthesizing and releasing amino acids during fermentation [14, 54].

These findings are consistent with previous studies by Enaemene et al. (2020), Arshad et al. (2023), and Rodríguez-España et al. (2022), highlighting the synergistic effects of germination and fermentation on protein quality [14, 40].

Table 3. Amino acid profiles (%) of different mung beans

| Amino acids | Treatments | | |
|---|---|---|---|
| | RMB | SMB | SFMB |
| Aspartic acid | 0.10$^{b}$ | 2.30$^{a}$ | 2.70$^{a}$ |
| Glutamic acid | 0.30$^{c}$ | 2.10$^{b}$ | 3.0$^{a}$ |
| Serine | 0.20$^{c}$ | 0.70$^{b}$ | 1.0$^{a}$ |
| Histidine | 0.30$^{c}$ | 0.60$^{a}$ | 0.50$^{b}$ |
| Glycine | 0.20$^{c}$ | 0.30$^{b}$ | 0.60$^{a}$ |
| Threonine | 0.20$^{c}$ | 0.60$^{b}$ | 0.70$^{a}$ |
| Arginine | 0.30$^{c}$ | 1.40$^{b}$ | 1.80$^{a}$ |
| Alanine | 0.04$^{c}$ | 0.30$^{b}$ | 0.60$^{a}$ |
| Tyrosine | 0.07$^{c}$ | 0.40$^{b}$ | 0.80$^{a}$ |
| Methionine | 0.06$^{c}$ | 1.20$^{b}$ | 1.30$^{a}$ |
| Valine | 0.04$^{c}$ | 0.30$^{b}$ | 0.50$^{a}$ |
| Phenylalanine | 0.20$^{b}$ | 0.70$^{a}$ | 0.70$^{a}$ |
| Isoleucine | 0.05$^{b}$ | 0.30$^{a}$ | 0.30$^{a}$ |
| Leucine | 4.70$^{c}$ | 22.30$^{b}$ | 40.8$^{a}$ |
| Lysine | 0.50$^{c}$ | 1.30$^{b}$ | 1.40$^{a}$ |

Different letters in each row indicate statistically significant differences at the 5% level. RMB: Raw mung bean sample, SMB: Sprouted mung bean sample, SFMB: Sprouted and fermented mung bean sample.

## 4-Conclusion

This study investigates the effects of sprouting and fermentation processes using Saccharomyces cerevisiae on the physicochemical properties and overall quality of mung bean seeds. The results showed that both fermentation and sprouting led to a significant reduction in moisture content and starch, while enhancing the levels of sugars, fats, and ash. Phenolic compound content and antioxidant activity were notably increased during fermentation, which can be attributed to the release of bound phenolic acids as a result of sprouting, thereby improving their bioavailability during the fermentation process. Additionally, the enzymatic actions involved in sprouting and fermentation contributed to a marked reduction in phytic acid content. The levels of water-soluble vitamins, particularly those from the B-vitamin family and vitamin C, were also significantly elevated through these processes. Furthermore, both sprouting and fermentation enhanced protein content and amino acid profile, an effect linked to yeast activity. The findings suggest that combining sprouting and fermentation processes can be a promising strategy to improve the nutritional value of mung beans. Future studies could explore the impact of varying treatment conditions, such as temperature, duration, and ultrasound treatment, on enhancing nutritional compounds and reducing antinutritional factors in legumes and cereals.

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# بررسی اثر فرآیند جوانه زنی و تخمیر به عنوان بهبود ارزش تغذیه ای دانه ماش

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**چکیده**

امروزه توجه به حبوبات جوانه زده در صنایع غذایی اهمیت فراوانی یافته است. در این پژوهش تاثیر فرایند جوانه‌زنی و فرآیند جوانه زنی و تخمیر توام با ساکارومایسز سروزیه بر خصوصیات فیزیکوشیمیایی، کیفی و تغذیه‌ای ماش مورد بررسی قرار گرفت. رطوبت، پروتئین، چربی، خاکستر، نشاسته، قند، اسید فیتیک، فنول کل و فعالیت آنتی اکسیدانی، محتوی ویتامین های محلول در آب و پروفایل اسیدهای آمینه نمونه های مختلف ماش اندازه گیری شد. نتایج نشان داد فرآیند تخمیر و جوانه زنی منجر به کاهش رطوبت (٦/٨٥ ٪) و نشاسته (۲۹/۸٤ ٪) و افزایش قند (۲/۲ میلی گرم بر میلی لیتر)، چربی (۲/۳۸ ٪) و خاکستر (۵/۲۷ ٪) شده است. میزان ترکیبات فنولی کل (۷۲/۰۱ میلی گرم اسید گالیک بر گرم خشک) و خاصیت آنتی اکسیدانی مهار رادیکال آزاد دی-پی‌پی‌اچش (۷٤/۳۲ ٪) با عملیات تخمیر افزایش یافت که به دلیل آزاد شدن فنول‌های باند شده در نتیجه فرآیند جوانه زنی و افزایش زیست دسترس پذیری آن ها در فرآیند تخمیر بود. تخمیر و جوانه زنی با استفاده از آنزیم های تجزیه کننده منجر به کاهش محتوی اسید فیتیک (۰/٦٤ میلی گرم بر ۱۰۰ گرم) شد. میزان ویتامین های محلول در آب با اعمال فرآیند تخمیر و جوانه زنی افزایش یافت. فرآیند جوانه زنی و تخمیر منجر به افزایش میزان پروتئین (۲۹/۸۳ ٪) و همچنین افزایش اسیدهای آمینه شد که مرتبط با فعالیت مخمر می‌باشد. نتایج این تحقیق استفاده از فرآیند جوانه زنی و تخمیر را به منظور افزایش ارزش تغذیه‌ای ماش مثبت ارزیابی نمود.