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# EVALUATION OF NUTRITIONAL AND PHYSICAL PROPERTIES OF PROBIOTIC-ENRICHED EXTRUDATES FROM SPROUTED LEGUME BEAN FLOUR

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**ABSTRACT**
The present study was conducted to evaluate the nutritional and physical characteristics of probiotic-enriched extrudates developed by using sprouted legume bean flours. Six different formulations were prepared using 100% sprouted and raw legume flours including white kidney beans, red kidney beans, mung beans, black gram and a composite blend. The physical attributes (color parameters L*, a*, b* and texture) and proximate composition of the extrudates were assessed. Data was statistically analyzed. The texture values, moisture content, protein content and fat content of extrudates were ranged from 16.40±0.04 to 25.66±0.04N, 4.86±0.02 to 8.67±0.02%, 22.05±0.04 to 26.04±0.06%, respectively. Likewise mean values for viable cells of extrudates were ranged from 1.19±0.03 to 4.09±0.01logCFU/g. The study concludes that sprouted legume flours are suitable carriers for probiotic enrichment in extruded snacks, offering improved nutritional profiles and acceptable physical properties for functional food applications.

**Key Words:** Kindy beans, Mung beans, Probiotics, Extrudates, Physical Properties

## 1. Introduction

Legumes, belonging to the family *Leguminosae*, are the consumable seeds of leguminous plants, comprising 600 genera and approximately 13,000 species Ahmed (2023). The word legumes denotes a category of prevalent legumes, encompassing the mung beans (*Vigna Radiata*), red kidney beans (*Phaseolus vulgaris* L.), white kidney beans (*Phaseolus vulgaris* L.) and black gram beans (*Vigna mungo*). Worldwide, legumes occupy 12-15% of fertile land to produce 27% of total crop production.

The legume beans cultivated in many parts of the world, including Asia, Africa, and America. Regionally, Asia leads in legume beans production with about 43% of global production, followed by America 29% and Africa 26% (Uebersax *et al.,* 2022).

Mung bean is one of the most important annual, warm seasonal and edible legume crops, which is most widely cultivated in Pakistan, Afghanistan, Iran and Australia and known as green grams or green pearls (Mohammad *et al.,* 2024). The total production of mung bean are 135 MT in Pakistan (Pakistan Economic Survey 2022-2023). Mung beans contain balanced macro and micro nutrients, including proteins, dietary fibers, vitamins and minerals, as well as high amounts of bioactive components, fatty acids, including linoleic acid and linolenic acid (Mbeyagala *et al.,* 2023).

Red kidney beans is a vital leguminous crop produce globally also known as red beans, because of their reddish-brown coloring and kidney-shaped form, kidney beans are also known as red beans or common beans (Fatima and Sajjad, 2023). Red kidney beans production is commonly seen in the Himalaya belt due to favorable climate conditions for beans cultivation. Pakistan well grown red kidney beans in tropical and subtropical regions. The consumption of red kidney beans is associated with many health benefits that can fight against chronic non-communicable diseases such as diabetes, cancer, obesity and coronary heart disease.

Red kidney beans exhibit superior amino acid profile, predominantly abundant in lysine, leucine, aspartic acid and glutamic acid. The predominant carbohydrate in red kidney bean seeds is starch, constituting 25-35% of the dry content. Red kidney beans include 8.85-10.2% moisture, 22.9-26.3% protein, 3.80-7.00% fiber and 3.1-4% ash (Enyiukwu *et al.,* 2020). The red kidney beans comprises a substantial quantity of phytochemical substances, including phenolics and flavonoids (Sudhakaran and Bukkan, 2021). The sprouting process of red kidney beans activates the enzymes, including protease, lipase and carbohydrates (Winarsi *et al.,* 2020).

White kidney beans is one of the most globally important legume crops and an important component of human nutrition because of high protein content 20-25%, complex carbohydrates 50-60% and starch 25%-45% (Siva *et al.,* 2020). White kidney beans include minerals 3.5%, fat 1% and crude fiber 5.1% (Winarsi *et al.,* 2020). White kidney beans also contains an adequate quantity of oligosaccharides like raffinose, stachyose, verbascose and ajugose that act as substantial prebiotic components and provide the matrix for probiotic microorganisms for grow (Sudhakaran and Bukkan, 2021).

Black gram beans is a member of the family *Leguminosae,* also locally known as Urd bean and Mash. Black gram rich in proteins, minerals, fibers and bioactive compounds with numerous pharmacological properties (Saeed *et al.,* 2020). However, it is estimated that black gram is the major food legume grown in Pakistan during the summer season occupies 1.5% of total legume area contributing 1.4% to total legume production. Black gram was grown over an area of 17 (000 hectares) with a total production of 7 MT in Pakistan (GOP 2022). Black gram used in various food items such as snacks, baked goods, drinks and fermented foods (Pasquale *et al.,* 2020). Black gram have potential health effects include antidiabetic, anti-obesity, antioxidant and anti-inflammatory properties (Smriti and Snehasis, 2022).

Various processing methods, including cooking, microwave cooking, pressure cooking, fermentation, sprouting and extrusion can be applied to legume beans to reduce anti-nutritional factors and enhance their digestibility and nutritional value (Wiesinger *et al.,* 2022). The sprouting of legume beans acts as an inexpensive and effective processing method that increases the quantity and availability of essential nutrients and reduces the level of anti-nutrients. Sprouting of legumes occurs optimal nutrient utilization arises from the catabolism of macronutrients into smaller molecules, including amino acids, simple sugars and other nutritious components (Ohanenye *et al.,* 2020).

The consumption of sprouts has increased, as considered a functional food that improves the health status of the consumer in recent year. The intake of sprouts is prevalent in Asia and Western countries, where sprouts are typically regarded as a component of good health. The sprouts can be ingested in both raw and cooked forms. Legume sprouts can be consumed in different form such as salads, sauces, wraps and soups. Some sprouts especially mung bean can be blended in smoothies for an extra nutritional health benefits Sadaf (2023).

Probiotics are defined as *live microorganisms which when administered in adequate amounts confer a health benefit on the host.* Food based probiotic products account for a large number of probiotic formulations and can be divided in two distinct categories, dairy products e.g. cheeses, yogurts, ice cream and milk and non-dairy products e.g. meats and meat products, bread or other fiber snacks, chocolates, fruit juices and fruit preparations (Trabelsi *et al.,* 2019).

Prebiotics are compounds in food that stimulate growth of beneficial microorganisms such as bacteria and fungi. Prebiotics are non-digestible food components, mainly fibers that promote the growth and activity of beneficial bacteria probiotics. Prebiotic consumption has also been demonstrated to increase the quantity of good bacteria in the gastrointestinal tract in a specific way. Natural sources of prebiotics are abundant in various foods including fruits, vegetables, cereals, milk and honey etc. Recent studies have highlighted the potential of several legumes to serve as valuable sources of prebiotics (Valerocases *et al.,* 2020).

Sprouting of legumes for producing nondairy source of probiotics

Development of sprouted legumes based extrudates

Investigation of extrudates for their physical, nutritional properties and evaluated probiotics viable cells

## 2. Materials and Methods

Present study was intend to development of encapsulated extrudates by using raw and sprouted mung bean, black gram beans, white kidney beans and red kidney beans flour in different combinations. Extrudates manufacturing trails was performed and analysis were performed in the Food Technology Lab, Food Processing and Preservation Lab and Food Analytical Lab, Department of Food Science and Technology, Govt College Women University, Faisalabad.

### 2.1. Procurement of raw materials

The procurement of raw material such as mung beans, black gram beans, white kidney beans and red kidney beans were purchased from Ayub Agriculture Research Institute, Faisalabad and Nuclear Institute of Agriculture and Biology, Faisalabad.

### 2.2. Preparation of raw legume beans flour

Raw legume beans (mung beans, black gram beans, white kidney beans and red kidney beans) were properly cleaned to remove stones, dirt and other extraneous matters before being used for further processing. Cleaned raw legume beans were washed, dried and ground into flour, packed in airtight jars and stored for further used.

### 2.3. Development of sprouted legume beans flour

Legume beans (mung beans, black gram beans, white kidney beans and red kidney beans) were disinfected in 1% (v/v) sodium hypochlorite for 10 min, drained, and washed with distilled water until they reached a neutral pH (7). Legume beans were soaked in distilled water for simple sprouting and socked into *Lactobacillus plantarum* DMS 9843 (299V) water suspension (1 × 108 CFU per 1g of seeds) for probiotics enriched sprouting. The legume beans were soaked for 6 and 8 h respectively. The legume beans (approximately 12g per plate) were dark-germinated for 4 days in a growth chamber (MLR-350H, Sanyo, Japan) on petri dishes (φ 125mm) lined with absorbent paper (relative humidity 90%). Seedlings were sprayed daily with 5mL of Milli-Q water. Sprouting was carried out at 25 °C. Four-day-old legume bean sprouts were manually collect and immediately frozen trough freeze-dried and stored ground into flour, packed in airtight jars and stored (Swieca *et al.,* 2020).

**Table 1: Treatments plan for development of sprouted legumes based extrudates**

| Treatments | Raw beans flour (WKB, RKB, MB, BGB %) | Sprouted beans flour (WKB, RKB, MB, BGB %) |
|---|---|---|
| $T_0$ | 100(25%+25%+25%+25%) | 0 |
| $T_1$ | 0 | 100(25%+25%+25%+25%) |
| $T_2$ | 0 | 100 (WKB) |
| $T_3$ | 0 | 100 (RKB) |
| $T_4$ | 0 | 100 (MB) |
| $T_5$ | 0 | 100 (BGB) |

*WKB (White Kidney Beans) *RKB (Red Kidney Beans) *MB (Mung Beans) * BGB (Black Gram Beans)

**2.4. Development of extrudates**

The development of extrudates was prepared by the method of Bilal *et al.* (2021) with slight modification. The samples was fed to the extruder barrel through a conical hopper of a co-rotating twin screw extruder. The rotational speed was 300rpm and diameter of die was 6mm. The temperature of the barrel sections was set at 65 °C for first section, 75 °C for second section and 100 °C for third section. The first section of extruder is the feeding section where proper mixing of the ingredients were takes place. The second section (compression section), pressure is built up due to increase in temperature by either friction or heating coils. The third section final transformation takes place, where the mixture forms a homogeneous viscoelastic mass. The transitions in this section control the texture, structure, color and flavour of the final product. Expansion of the product occurs right after this section at the die (exit) due to pressure and temperature drop. The extrudates was collected and cooled to room temperature before packing in laminated pouches and stored for analysis.

**2.4.1. Analysis of extrudates**

**2.4.1.1. Physical analysis of extrudates**

**2.4.1.2. Color analysis of extrudates**

The colorimeter was used to evaluate the color parameters (L *, a *, b *) where L * indicates lightness from black (L=0) to white (L=100) a * indicates green (-a *) to red (+ a *) and b * indicates blue (-b *) to yellow (+ b *) axis. To measure the color of raw, sprouted and encapsulated legumes based extrudates were carefully sliced with a knife. For the measurement of L *, a * and b * values, the hue angle (ho), chroma (C) and the total color difference between the control and other samples were measured (Cihan and Masatcioglu, 2022).

**2.4.1.3. Texture analysis of extrudates**

The texture analysis of raw, sprouted and encapsulated sprouted legumes based extrudates was determined using a texture analyzer according to the procedure Cihan and Masatcioglu, (2022) with slight modification. Before the texture analysis samples of extrudates were properly dried and approximately 5cm long extruded breakfast cereals were placed in the center of the texture analyzer plate. The hardness was measured by taking absolute peak force.

**2.4.2. Proximate analysis of legumes based extrudates**

**2.4.2.1. Moisture content**

The moisture content of was determined by the method as described AACC (2019) using hot air oven at temperature 105 °C for 6-7 hrs AACC 2019).

**2.4.2.2. Fat content**

The fat content of was determined by the use of Hexane as a solvent in a soxhlet apparatus as described in AACC (2019).

**2.4.2.3. Protein content**

The protein content was determined according to the procedure given in AACC (2019).

**2.4.2.4. Fiber content**

The fiber content of was estimated by taking a fat-free sample as termed in AACC (2019) method 985.29.

**2.4.2.5. Ash content**

The ash content of extruded breakfast cereals was estimated through the following procedure as given in AACC (2019).

**2.4.2.6. Nitrogen free extract (NFE)**

The nitrogen-free extract was determined by following formula:

NFE (%) = 100 – (Moisture % + Crude fat % + Crude protein % + Crude fiber % + Crude ash %)

**2.4.3. Probiotics viable cells of extrudates**

The probiotics viable cells of extrudates was estimated as per the study by (Smriti and Snehasis, 2022).

**2.4.4. Statistical analysis**

The data was statistically analyzed by 8.1 software by applying latin square design and mean values were calculated (Montgomery *et al.,* 2013).

**3. Results and Discussion**

**3.1. Analysis of extrudates**

**3.1.1. Physical analysis of extrudates**

**3.1.1.1. Color (L*, a*, b*) of extrudates**

The mean squares of color (L*, a*, b*) and texture (hardness) of raw, sprouted and encapsulated sprouted legume based extrudates have been depicting in Table 1. The analysis of variance regarding color (L*, a*, b*) exhibited significant results $p \leq 0.05$ whereas texture showed highly significant result $p \leq 0.01$. The mean values regarding color (L*, a*, b*) have been revealed in Table 2. The luminosity (L*) values of extrudates were ranged from 35.11±0.10 to 35.11±0.10.

The results regarding L* value correlated with previous study by Marchioni *et al.* (2021) who was observed L* value of sprouted cereals to produce healthier extrudates are 34.17±0.46 to 61.23±1.41. The lightness value (L*) was highest for treatment $T_3$ (44.27), which contained 100% sprouted red kidney beans flour**,** indicating that this flour contributed to a brighter appearance of the extrudates. The high L* value might be attributed to the color characteristics of red kidney beans, which, after sprouting, undergo biochemical changes that may reduce pigment concentration and enhance brightness. Conversely, the lowest L* value was observed in $T_0$ (35.11), made from 100% raw beans flour (a mix of four legumes**)**. This suggests that raw flours, compared to sprouted ones, may retain more natural pigments or anti-nutritional compounds that contribute to a darker appearance due to less enzymatic breakdown.

The a* values of extrudates were ranged from 2.23±0.03 to 3.35±0.05. The b* values of extrudates have been ranged from 19.36±0.07 to 24.54±0.04. Lorena *et al.* (2024) was observed a* value were ranged 0.79 ± 0.09 to 5.3 ± 0.1 and b* value were ranged from 37.5 ± 0.7 to 55.05 ± 0.46 of cornmeal extrudates incorporated with carotenoid-rich extract from pumpkin by-product. For redness (a*), the highest value was seen in $T_0$ (3.35), while the lowest was in $T_4$ (2.23), composed of 100% sprouted mung bean flour. The higher a* in $T_0$ could be due to the presence of naturally pigmented compounds in raw legume flours. Sprouting generally leads to degradation of these pigments, hence a reduction in redness, especially in mung beans, which are naturally lighter in color.

In terms of yellowness (b*), $T_4$ again showed the highest value (24.54), indicating the strong contribution of mung beans to yellowness in extrudates. This could be due to the enhanced visibility of yellow pigments post-sprouting. On the other hand, $T_0$ exhibited the lowest b* value (19.36), reinforcing the observation that sprouting enhances color attributes by modifying pigment profiles and improving color clarity.

### 3.1.1.2. Texture analysis of extrudates

The mean squares of texture (hardness) of extrudates have been depicting in Table 1. The analysis of variance regarding texture exhibited significant results p≤0.05. The mean values regarding texture have been revealed in Table 2. The texture values of extrudates were ranged from 16.40±0.04 to 25.66±0.04N. Sudhakar *et al.* (2021) reported that an increase in fibre amount resulted in increased cell wall thickness and texture hardness of extrudates. exture analysis revealed that the highest texture value (25.66 N) was found in $T_5$, which used 100% sprouted black gram beans flour**.** The firmer texture might be due to the higher protein and starch interaction after sprouting, which supports stronger structural development during extrusion. In contrast, $T_2$, prepared with 100% sprouted white kidney beans flour, had the lowest texture value (16.40 N), indicating a more brittle or less dense structure. This may be due to lower starch-protein interaction or a less favorable composition for forming a rigid matrix during extrusion.

**Table 1: Mean squares for physical analysis color (L*, a*, b*) and texture of legume based extrudates**

| SOV | Df | MS | | | |
|---|---|---|---|---|---|
| | | **L*** | **a*** | **b*** | **Texture (N)** |
| Treatments | 5 | 25.8035** | 1.32085** | 33.4375** | 32.1888* |
| Error | 11 | 0.0097 | 0.00313 | 0.0481 | 0.0018 |
| Total | 16 | | | | |

*Significant P≤ 0.05, **Highly Significant P≤0.01

**Table 2: Mean values for physical analysis color (L*, a*, b*) and texture of legume based extrudates**

| Treatments | L* | a* | b* | Texture (N) |
|---|---|---|---|---|
| $T_0$ | $35.11\pm0.10^f$ | $3.35\pm0.05^a$ | $19.36\pm0.07^f$ | $24.15\pm0.04^e$ |
| $T_1$ | $40.28\pm0.24^e$ | $3.19\pm0.17^c$ | $20.45\pm0.05^e$ | $18.25\pm0.03^d$ |
| $T_2$ | $39.30\pm0.03^d$ | $2.70\pm0.03^f$ | $23.21\pm0.01^b$ | $16.40\pm0.04^f$ |
| $T_3$ | $44.27\pm0.24^a$ | $3.12\pm0.03^e$ | $21.34\pm0.04^d$ | $23.33\pm0.05^c$ |
| $T_4$ | $42.34\pm0.04^b$ | $2.23\pm0.03^d$ | $24.54\pm0.04^a$ | $25.61\pm0.04^b$ |
| $T_5$ | $43.14\pm0.04^c$ | $3.33\pm0.03^b$ | $22.54\pm0.04^c$ | $25.66\pm0.04^a$ |

$T_0$=100% (25%+25%+25%+25%) raw beans flour
$T_1$=100% (25%+25%+25%+25%) sprouted beans flour
$T_2$=100% sprouted white kidney beans flour
$T_3$=100% sprouted red kidney beans flour
$T_4$=100% sprouted mung beans flour,
$T_5$=100% sprouted black gram beans flour

### 3.1.2. Proximate composition of extrudates

Mean values for moisture, fat and protein content of extrudates have been displayed in Table 3. Analysis of variance of moisture content of extrudes represents highly significant results. Mean values for moisture content of extrudates have been showed in Table 4. The mean values for moisture content of extrudates were ranged from 4.86±0.02 to 8.67±0.02%. Results regarding moisture content of extrudates agreed with the findings of Ratchaneeporn *et al.* (2023) was found moisture content of cereal based extruded snacks ranged from 4.55 to 8.04% in puffed snacks. Mung beans are known for their higher water retention capacity, especially after sprouting, due to enhanced enzymatic activity and breakdown of complex carbohydrates, which improves water-binding properties. In contrast, the lowest moisture content was observed in $T_0$ (4.86%), the treatment made from 100% raw beans flour. The lower moisture in raw flour extrudates could be due to limited hydration and lack of biochemical changes that occur during sprouting, which restricts water absorption and retention during processing.

The mean values for fat content of extrudates were ranged from 1.17±0.02 to 5.27±0.02%. Fat content was highest in $T_3$ (5.27%), containing 100% sprouted red kidney beans flour**.** Red kidney beans may have naturally higher lipid content retain more oil during extrusion due to structural changes induced by sprouting, which can impact fat-binding properties. On the other hand, the lowest fat value was recorded in $T_2$ (1.17%), prepared with 100% sprouted white kidney beans flour. This could be attributed to lower inherent fat content in white kidney beans reduced ability to retain fat during extrusion, possibly due to a less dense matrix formation after sprouting. The results regarding the crude fat content concur with reported by EL-Suhaibani *et al.* (2020) on goat pea which reduced from 1.88% raw to 1.43% upon sprouting.
The mean values for protein content of extrudates were ranged from 22.05±0.04 to 26.04±0.06%. The minimum crude protein content was observed in $T_{10}$ (97% sprouted white kidney beans and 3% okra gum) 25.58±0.03%. Protein content was highest in $T_0$ (26.04%), the extrudate made from raw mixed legume flours. The high protein value in raw flour could be due to the preservation of native protein structures that might degrade slightly during sprouting or due to variations in protein solubility post-sprouting. The lowest protein content was noted in $T_2$ (22.05%), suggesting that sprouted white kidney beans might have a lower protein content experience more protein breakdown during sprouting and subsequent extrusion. Protein degradation during sprouting is common due to increased protease activity, which may lead to partial hydrolysis of storage proteins. The rise in protein could result from hydrolysis of seed proteins and from decrease of certain antinutrients Okpala and Kanu, (2023).
Mean values for fiber, ash and NFE content of extrudates have been displayed in Table 5. Analysis of variance of fiber content of extrudes represents significant results. Mean values for fiber, ash and NFE content of extrudates have been showed in Table 6. The mean values for fiber content of extrudates were ranged from 22.05±0.04 to 26.04±0.06%. Crude fiber content was highest in $T_2$ (8.17%), which consisted of 100% sprouted white kidney beans flour. The increase in fiber content in this treatment could be due to the breakdown of complex carbohydrates during sprouting, which exposes and enhances the measurable fiber fractions. White kidney beans are also naturally high in dietary fiber and sprouting can further enhance fiber availability. In contrast, the lowest fiber content was observed in $T_0$ (4.16%), made from 100% raw mixed legume flours. The low fiber in $T_0$ attributed to the lack of enzymatic modifications that typically occur during sprouting, resulting in less exposure and accessibility of fiber components. Raw legumes contain anti-nutritional compounds that interfere with fiber extraction and utilization (Wongsiri *et al.,* 2020).
The mean values for ash content of extrudates were ranged from 3.44±0.02 to 4.49±0.03%. Sprouting enhances the bioavailability of minerals by reducing anti-nutritional factors such as phytic acid, which can otherwise bind to minerals and reduce their availability. This process leads to a higher concentration of ash, which represents the total mineral content in the extrudates (Luthria *et al.,* 2020). Ash content, which reflects the total mineral content, was found to be highest in $T_5$ (4.49%), prepared with 100% sprouted black gram beans flour. The increase in ash may be due to improved mineral bioavailability and concentration after sprouting, as sprouting tends to reduce antinutritional factors like phytates that normally bind minerals. On the other hand, the lowest ash value was recorded in $T_2$ (3.44%), indicating that despite its high fiber content, sprouted white kidney beans flour possess comparatively lower mineral content or reduced ash accumulation during extrusion.
The mean values for NFE content of extrudates were ranged from 3.44±0.02 to 4.49±0.03%. Sprouting activates enzymes such as amylases, which hydrolyze complex carbohydrates into simpler sugars. Nitrogen-Free Extract (NFE), which represents the digestible carbohydrate fraction, was highest in $T_0$ (58.53%), the treatment made from raw mixed legume flours. This high value can be attributed to the intact starch and sugar contents in raw flours, which are not partially hydrolyzed consumed during sprouting. Conversely, the lowest NFE was observed in $T_3$ (53.48%), made from sprouted red kidney beans flour. This enzymatic breakdown reduces the overall carbohydrate (starch) content while increasing other macronutrients such as proteins and fibers (Hafidh *et al.,* 2020).

**Table 3: Mean squares for moisture, fat and protein content of legume based extrudates**

| SOV | DF | Moisture | Fat | Protein |
|---|---|---|---|---|
| Treatments | 5 | 6.59767** | 8.29768** | 9.43037** |
| Error | 11 | 0.00112 | 0.00079 | 0.00151 |
| Total | 16 | | | |

**Highly Significant P≤0.01

**Table 4: Mean values for moisture, fat and protein content of legume based extrudates**

| Treatments | Moisture % | Fat % | Protein % |
|---|---|---|---|
| $T_0$ | 4.86±0.02$^f$ | 2.90±0.02$^b$ | 26.04±0.06$^a$ |
| $T_1$ | 7.99±0.03$^c$ | 2.24±0.04$^c$ | 24.70±0.02$^c$ |
| $T_2$ | 8.04±0.04$^b$ | 1.17±0.02$^f$ | 22.05±0.04$^f$ |
| $T_3$ | 7.08±0.04$^e$ | 5.27±0.02$^a$ | 23.26±0.03$^e$ |
| $T_4$ | 8.67±0.02$^a$ | 1.33±0.11$^d$ | 24.53±0.02$^d$ |
| $T_5$ | 7.83±0.03$^d$ | 1.19±0.05$^e$ | 25.03±0.02$^b$ |

$T_0$=100% (25%+25%+25%+25%) raw beans flour
$T_1$=100% (25%+25%+25%+25%) sprouted beans flour
$T_2$=100% sprouted white kidney beans flour
$T_3$=100% sprouted red kidney beans flour
$T_4$=100% sprouted mung beans flour,
$T_5$=100% sprouted black gram beans flour

**Table 5: Mean squares for fiber ash and NFE of extrudates**

| SOV | DF | Fiber | Ash | NFE |
|---|---|---|---|---|
| Treatments | 4 | 6.64301* | 0.34347* | 7.5544** |
| Error | 10 | 0.0007 | 0.0006 | 0.0006 |
| Total | 14 | | | |

*Significant P≤ 0.05, **Highly Significant P≤0.01

**Table 6: Mean values for proximate composition of extrudates**

| Treatments | Crude Fiber % | Crude Ash % | NFE % |
|---|---|---|---|
| $T_0$ | 4.16±0.03$^f$ | 3.53±0.03$^d$ | 58.53±0.02$^a$ |
| $T_1$ | 5.86±0.02$^b$ | 3.90±0.02$^c$ | 55.33±0.01$^d$ |
| $T_2$ | 8.17±0.02$^a$ | 3.44±0.02$^f$ | 57.06±0.02$^b$ |
| $T_3$ | 5.57±0.02$^c$ | 4.23±0.02$^b$ | 53.48±0.03$^f$ |
| $T_4$ | 5.09±0.03$^d$ | 3.47±0.02$^e$ | 56.93±0.04$^c$ |
| $T_5$ | 4.73±0.02$^e$ | 4.49±0.03$^a$ | 54.91±0.02$^e$ |

$T_0$=100% (25%+25%+25%+25%) raw beans flour
$T_1$=100% (25%+25%+25%+25%) sprouted beans flour
$T_2$=100% sprouted white kidney beans flour
$T_3$=100% sprouted red kidney beans flour
$T_4$=100% sprouted mung beans flour,
$T_5$=100% sprouted black gram beans flour

### 3.1.3. Probiotic viable cells of extrudates

Mean values for viable cells of extrudates have been displayed in Table 7. Analysis of variance of viable cells of extrudes represents highly significant results. Mean values for viable cells of legumes based extrudates have showed in Table 8. The probiotic viable cell count (log CFU/g) varied significantly among different treatments, indicating the influence of formulation components on probiotic survival. The mean values for viable cells of extrudates were ranged from 1.19±0.03 to 4.09±0.01logCFU/g. The maximum probiotic viable cells in extrudates was observed in $T_1$ 100%

(25%+25%+25%+25%) 4.09±0.01logCFU/g the highest probiotic count among the base treatments, suggesting that its composition provided a favorable environment for probiotic growth and stability (Saputro *et al.,* 2023).

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**Table 7: Mean square probiotic viable cells of extrudates**

| SOV | DF | Probiotic viable cells |
|---|---|---|
| Treatments | 5 | 1.0348** |
| Error | 11 | 0.4681 |
| Total | 16 | |

**Highly Significant P≤0.01

**Table 8: Mean values for probiotic viable cells of extrudates**

| Treatments | Probiotic viable cells logCFU/g |
|---|---|
| $T_0$ | Not Applicable |
| $T_1$ | 4.09±0.01[a] |
| $T_2$ | 2.15±0.02[d] |
| $T_3$ | 2.33±0.03[c] |
| $T_4$ | 3.29±0.01[b] |
| $T_5$ | 1.19±0.03[f] |

$T_0$=100% (25%+25%+25%+25%) raw beans flour
$T_1$=100% (25%+25%+25%+25%) sprouted beans flour
$T_2$=100% sprouted white kidney beans flour
$T_3$=100% sprouted red kidney beans flour
$T_4$=100% sprouted mung beans flour,
$T_5$=100% sprouted black gram beans flour