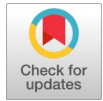


The Dynamic Role of Ultrasonic Treatment Technology in Flax Seed for the Development of Fortified Mushroom Powder to Boost Nutritional Benefits in Drink Development

Kajal Verma, Prity Pant, R C Mishra, Syed Samar Abbas



Abstract: This study investigated the physicochemical properties and sensory acceptability of fortified mushroom smoothies using blends of *Agaricus bisporus* with chia, flax, and pumpkin seeds. Standard analytical techniques and Pearson's correlations were used to examine blends containing different levels of mushroom flour (10%, 15%, 20%, 25%, and 30%). The findings revealed that increasing the mushroom content led to higher levels of protein, ash, fiber, zinc, and iron in both the composite flours and fortified smoothies. Additionally, the *in vitro* protein digestibility of the fortified smoothies improved with increased mushroom content. Adding *P. ostreatus* flour resulted in higher pH and lower total titratable acidity (TTA), whereas *A. bisporus* flour had the opposite effect. Moreover, higher mushroom content decreases fat, carbohydrates, and energy in the flours and smoothies, as well as reducing the viscosity of the fortified smoothies. Positive linear effects on foaming capacity, foam stability, fat absorption capacity, water retention capacity, water absorption capacity, solubility index, and swelling capacity were observed. At the same time, compact density, bulk density, and syneresis showed negative linear effects. The gelation capacity, emulsifying activity, and emulsion stability remained unaffected by *P. ostreatus* but slightly decreased with *A. bisporus*. The study highlights the potential of mushroom flours as a novel food fortification, given their nutritional benefits and numerous health-promoting attributes. (R.M. Ishara Jackson¹, Daniel N. Sila², Glaston M. Kenji²) (2018) [1].

Keywords: Mushroom Powder, TTA, Mushroom Content

I. INTRODUCTION

The global population continues to grow, leading to increased hunger and malnutrition in certain regions, exacerbated by the declining nutritional value of commonly consumed foods due to nutrient-depleted soils.

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The widespread malnutrition and the growing protein gap in developing countries have prompted the search for alternative protein sources. The production of pulses and cereals has not kept pace with the demands of a growing population, necessitating the exploration of nutritionally rich food sources that can support good health and enhance disease immunity. (Longvah, T.; Deosthale, Y.G.) (1998) [5]. The FAO has recommended edible mushrooms as a valuable food source that contributes to protein nutrition, especially for developing countries that rely heavily on cereals. Mushrooms are rich in proteins, carbohydrates, vitamins, fibers, and essential minerals and are considered safe for human consumption when commercially grown.

Mushrooms, or toadstools, are the fleshy, spore-bearing fruiting bodies of fungi, typically produced above ground on soil or their food source. The standard reference for "mushroom" is the cultivated white button mushroom, *Agaricus bisporus*. Thus, the term "mushroom" generally refers to fungi (Basidiomycota, Agaricomycetes) that feature a stem (stipe), a cap (pileus), and gills (lamellae) on the underside of the cap. These gills produce microscopic spores that aid in the fungus's dispersion across the ground or its substrate. During peak harvest season, the market quickly becomes saturated, forcing growers to resort to distress sales. As a result, unsold mushrooms become a total loss. To mitigate this, it is essential to develop methods for producing processed mushroom products. These products would not only reduce losses but also increase growers' income through value addition and improved marketing of this horticultural crop. Implementing appropriate post-harvest technologies to process surplus mushrooms into novel value-added products can help manage market gluts during peak harvesting periods. Mushroom protein, which is of intermediate quality between vegetable and animal proteins, holds considerable significance as a supplementary protein source in vegetarian diets.

Mushrooms are commonly eaten cooked or raw and used as garnishes in meals. They have a long history of use in traditional Oriental medicine, but their potential as "health potentiators" and "elicitors of the immune system" has only recently been recognized. Mushrooms are a major source of potent new pharmaceutical products and have gained attention as functional foods and sources for drug and nutraceutical development due to their antioxidant, antitumor, and antimicrobial properties.



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The active constituents in mushrooms include polysaccharides (β -glucans), dietary fibers, oligosaccharides, triterpenoids, peptides and glycoproteins, proteins, alcohols, phenols, and various minerals such as zinc, copper, iodine, iron, calcium, phosphorus, potassium, and selenium, as well as vitamins and amino acids.

In recent years, the growing affluence in developing countries has led to a significant increase in mushroom consumption due to their high nutritional value (Bano, Z.; Rajarathnam, S. *Pleurotus mushrooms*) 2021) [8][12]. Mushrooms are low-calorie foods, rich in protein, fiber, vitamins, and minerals, with low carbohydrate, fat, and calorie content (Mattila, P.; Konko, K.; Euvola, M.; Pihlava, J.; Astola, J.; Vahteristo, L) (2001) [7]. Table no. 1 provides the nutritional composition of mushroom powder. They are widely used in various cuisines, notably Chinese, Korean, European, and Japanese. Most mushrooms sold in supermarkets are commercially grown on mushroom farms, with *Agaricus bisporus* being the most popular. Several varieties of *A. bisporus*, including white, crimini, and Portobello, are grown commercially. Other cultivated species available at many grocery stores include shiitake, maitake (hen-of-the-woods), oyster, and enoki mushrooms.

The increasing interest in mushroom cultivation, particularly in developing countries, has turned it into a potentially important economic activity for small farmers. Some mushrooms or their extracts are used or researched as potential treatments for diseases such as cardiovascular disorders (Guillamón, E.; García-Lafuente, A.; Lozano) (2010) [4]. Certain mushroom components, including polysaccharides, glycoproteins, and proteoglycans, are under basic research for their potential to modulate immune system responses and inhibit tumor growth. Other isolates have shown potential antiviral, antibacterial, antiparasitic, anti-inflammatory, and antidiabetic properties in preliminary studies. Historically, mushrooms have been considered medicinally valuable, especially in traditional Chinese medicine. They have been studied in modern medical research since the 1960s, where most studies use extracts, rather than whole mushrooms. (Stamets, P.) (2005) [9]. The primary economic importance of mushrooms is their use as a food source for human consumption. Oyster mushrooms are rich in vitamin C and B complex vitamins, with a protein content ranging between 1.6 to 2.5 percent. They also provide essential mineral salts needed by the human body. Notably, the niacin content in oyster mushrooms is about ten times higher than that found in most other vegetables.

The folic acid in oyster mushrooms helps treat anemia. Due to their low sodium-to-potassium ratio, and minimal starch, fat, and caloric content, they are suitable for individuals with hypertension, obesity, and diabetes. Their alkaline ash and high fiber content make them beneficial for those with hyperacidity, constipation, and high cholesterol. Mushrooms are one of the few vegan sources of vitamin D and conjugated linoleic acid. They possess antioxidant properties due to compounds like ergothioneine. Oyster mushrooms can grow at moderate temperatures ranging from 20 to 30°C and a humidity of 55-70% for 6 to 8 months a year. They can also be cultivated during the summer by providing extra humidity, especially in hilly areas above 900 meters. The best growing season in these areas is from March/April to

September/October, while in lower regions, it is from September/October to March/April.

Mushrooms and chia seeds, as well as flax seeds, are healthy foods that complement each other exceptionally well. The concept of fortified mushroom integration is based on the idea that food influences our health in complex and interactive ways. Fortified Mushroom smoothies can be developed by combining the nutritional and health benefits of mushrooms with those of flax seeds, chia seeds, and pumpkin seeds. These smoothies are particularly beneficial for celiac patients, as they contain slow-releasing carbohydrates and are rich in antioxidants.

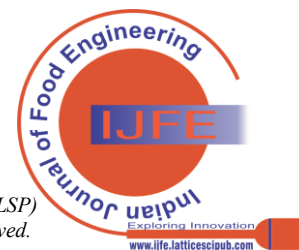
Mushrooms are valued as nutraceutical foods due to their significant functional and nutritional benefits. (Chang, S.T.; Miles, P.G. *Mushrooms*) (2008) [2]. They have garnered attention not only for their economic importance but also for their sensory appeal and medicinal properties. Distinguishing between medicinal and edible mushrooms can be challenging, as some medicinal varieties are edible, and many commonly consumed edible mushrooms offer therapeutic potential. The most widely cultivated mushroom is *Agaricus bisporus*, followed by *Flammulina velutipes*, *Lentinus edodes*, and various *Pleurotus* species. The crude protein content of edible mushrooms is typically high but varies widely depending on factors such as species and developmental stage. Edible mushrooms generally have low levels of free amino acids, typically ranging from 7.14 to 12.3 mg g⁻¹ in dry weight, which contributes significantly to their flavor profiles (Maga) (1981) [6]. However, they are deficient in sulfur-containing amino acids like methionine and cysteine, while being relatively rich in threonine and valine.

Cultivated mushrooms are abundant sources of vitamins such as folates, niacin, and riboflavin. They particularly stand out for their high vitamin B2 content compared to most vegetables, making them valuable dietary sources of this vitamin. The folate present in mushrooms is primarily in the bioavailable form of folic acid. Additionally, cultivated mushrooms contain small amounts of vitamin B1, vitamin C, and trace amounts of vitamin B12. They have low levels of total soluble sugars but are rich in oligosaccharides. Carbohydrate content in edible mushrooms ranges widely from 35 to 70% by dry weight, varying across different species. Fatty acids in mushrooms typically range from 2 to 8%, with polyunsaturated fatty acids constituting over 75% of total fatty acids, contrasting with lower levels of saturated fatty acids. Palmitic acid is the predominant saturated fatty acid (Ergönül, P.G.; Akata, I.; Kalyoncu, F.; Ergönül) (2013) [3].

II. MATERIAL AND METHOD

The present research was carried out in the Department of Food Safety and Quality Laboratory, IGMPI, Satsang Vihar, Near the Old JNU Campus, New Delhi.

Procurement of raw materials and development of products: Fresh oyster mushroom was purchased from the Local Market.



The mushroom powder was prepared by oven drying method from fresh mushrooms (Flow chart 1 and Figure 1). Fortified mushroom smoothies were prepared in which whey protein is replaced with mushroom powder and is added along with the significant value of Flax Seeds, Chia Seeds, and Pumpkin Seeds in their powdered form.

Ultrasound technology relies on mechanical waves with frequencies higher than what humans can hear (above 16 kHz). These ultrasonic waves can be classified into Low-intensity and High-intensity groups based on their frequency. Low-intensity ultrasound, also known as non-destructive or high-frequency ultrasound, uses high-frequency (100 kHz - 1 MHz) and low-power (<1 W/cm²) ultrasonic technology. Analytical methods that use low-intensity ultrasound provide information about the physical and chemical properties of food products, such as acidity, ripeness, firmness, and sugar content (Mohammed & Alhajhoj, 2019) [11].

High-intensity ultrasound, also known as low-frequency or power ultrasound, employs high power levels (typically ranging from 10 to 1000 W/cm²) and low frequencies (16 to 100 kHz) to enhance the quality of food. This technique induces physical or chemical changes using its high power and low frequency (Mohammed & Alhajhoj, 2019) [11]. Ultrasound has also emerged as a promising cleaning technique. During ultrasonic cleaning, foulant removal occurs due to either chemical interactions between the foulants and radicals generated by the ultrasonic treatment in the liquid or mechanical effects created by the ultrasound in the liquid medium. The two most used systems are the ultrasonic bath and the probe system of the sonochemistry apparatus, which are widely accessible to chemists. Ultrasound offers clear advantages in terms of productivity, yield, and selectivity, providing faster processing times, higher quality, lower risk of chemical and physical harm, and environmental friendliness.

If liquid pressure is reduced too much, it can cause the liquid to burst and produce vapor bubbles, a phenomenon known as cavitation. The mechanism of ultrasonication is based on the cavitation theory. Bubbles can form in a liquid exposed to ultrasonic radiation through the following methods: (i) Cavitation nuclei can form rapidly under high-intensity, low-frequency ultrasound (20 kHz) due to inertia

effects. (ii) Under low-intensity, high-frequency ultrasound (greater than 50 kHz), cavities can also develop, but their rate of expansion is slower because expansion only marginally increases the cavity's surface area compared to compression. Once a cavity reaches a certain size, it can no longer effectively utilize the sound field's energy to sustain itself, and this size is determined by the ultrasound's frequency. Ultrasonic waves induce cavitation, allowing the solvent to properly enter the cell and initiate the extraction process. They also create macroturbulence, high-velocity particle collisions, and movement in cell wall particles or the microporous matrix. Directing an ultrasonic jet at solid surfaces enhances the contact area between liquid and solid surfaces, facilitating efficient extraction of bioactive components from the solvent.

The flour used to develop the fortified mushroom powder in the study was made by grinding flax seeds, that had undergone ultrasonic treatment. Depending on its intensity, ultrasound can be applied to various processes, including enzyme activation or deactivation, mixing and homogenization, emulsification, dispersion, preservation, stabilization, dissolution and crystallization, hydrogenation, meat tenderization, ripening, aging, and oxidation. It is also used as an adjuvant for solid-liquid extraction to enhance and accelerate the extraction of active ingredients from various matrices.

A. Ultrasonic Treatment of Flax Seeds

Flax seeds (30g) were treated in water (200ml) using a digital ultrasonic cleaner (tank size 300x155x150mm, model no: LMUC-6, frequency 40KHz, power 150W). To maintain the sample's temperature below 30 degrees Celsius during ultrasound processing, it was placed in a tub of frozen water. To prevent overheating, the processing was paused for 15 minutes every 1.2 hours. Flax seed samples were processed for 30 minutes, 1 hour, 1.5 hours, and 2 hours, respectively, to produce four separate batches (labeled T1, T2, T3, and T4). The ultrasonicated seeds were then dried and milled into whole-grain flax flour using a Bajaj grinder (model no. FX11(410524)). After processing, the samples were stored in airtight containers and kept in a refrigerator.



T1



T2



T3



T4

B. Pre-Treatment of Flax Seeds

Flax seeds were subjected to ultrasonic treatment in four trials, designated T1, T2, T3, and T4, at a constant temperature of 35°C and a frequency of 40 kHz. After seed Soaking, the water turns opaque and slightly viscous from the soluble fiber and gum found on the surface of the seeds. As the time for soaking increases, the more viscous substance

comes on the surface of the seed. Ultrasonic treatment and soaking enhance the activity of the enzyme phytase, which helps reduce anti-nutritional components and improves mineral availability.

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Therefore, soaking and ultrasonic treatment is an effective method for decreasing anti-nutritional components such as tannins, phytic acid, and saponins. Additionally, soaking and ultrasonic treatment also enhance the antioxidant and nutritional properties of flax seeds. From the four trials T4 was found to have more soluble fiber and gums in the pre-treatment process.



Fig. 1: Ultrasonic Machine

C. Organoleptic Evaluation

The developed products were assessed for organoleptic quality attributes using a nine-point Hedonic scale by a panel of 10 semi-trained judges.

Score	Scale Grade
1	Disliked immensely
2	Disliked exceptionally
3	Disliked more than a little
4	Disliked a little
5	Neither liked nor disliked
6	Liked a little
7	Liked more than a little
8	Liked exceptionally
9	Liked immensely

D. Nutritive Value

The developed products were analyzed for proximate principles, including moisture, crude protein, crude fat,

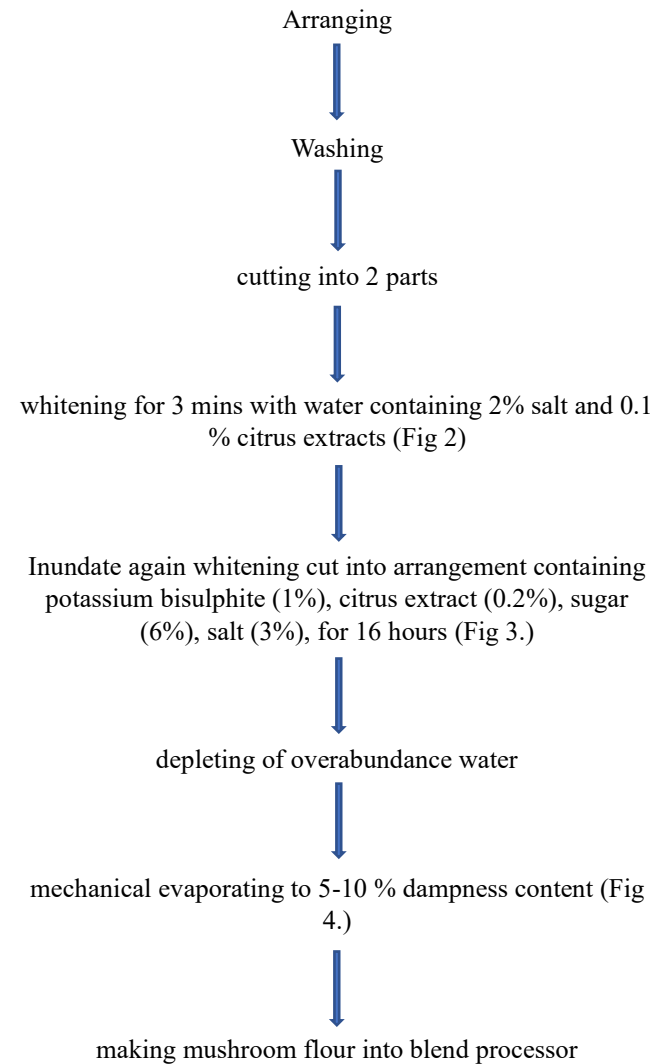
carbohydrate, total ash, and crude fiber, using standardized procedures from AOAC (1980).

E. Statistical Analysis

Analysis of variance (ANOVA) was conducted to analyze the data based on a completely randomized design. This was done to study the effect of mushroom powder fortification at different levels (10%, 15%, 20%, 25%, and 30%) on the sensory qualities and nutrient content of the prepared products.

- Salt
- Citrus extract
- Potassium bisulphite
- Sugar
- Oven
- Mixer Grinder
- flax seed powder
- Pumpkin seed powder
- Chia seed powder
- Honey
- mushroom powder.

F. Mushroom Flour: The Flow Diagram of Standard Procedure is as Follows



Flow Chart-1





Fig. 2: Cutting of Mushroom



Fig. 6: Dried Mushroom Flour



Fig 3: Dipping the Mushroom into the Saline Solution for 16 Hours



Fig. 7: Mixture of Mushroom Flour along with Chia Seeds, Flax Seeds and Pumpkin Seeds in the Blend Form



Fig. 4: Dipping the Mushroom into the Saline Solution for 16 Hours

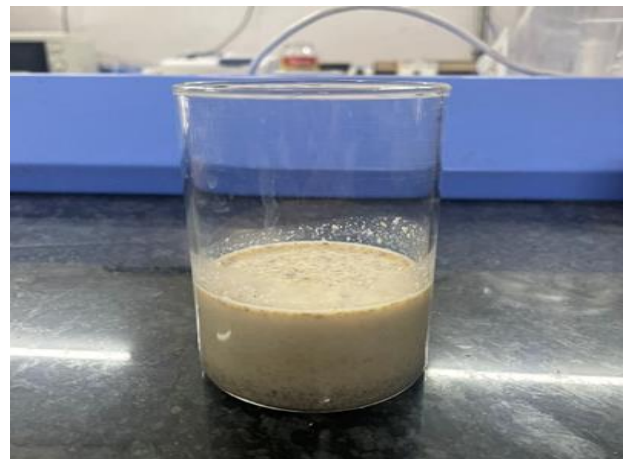


Fig. 8: Fortified Mushroom Product of Mushrooms Flour



Fig. 5: Drying the Mushroom into Oven for 6 Hours at 50°C

1. Mushroom Fortified Drink – Take 80 ml of low-fat milk then add 2.5 gm of Mushroom powder, flax seed powder (2.5 gm), Pumpkin seed powder (2.5 gm), Chia seed powder (2.5 gm). Mix all ingredients which will provide a healthy drink to meet up the daily requirement of Energy and diet of essential fatty acid which are not synthesis by human body. This drink will be very healthy for the children above 5 years of age.

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Table-1: Nutritive Value of Developed Mushroom Powder

S. No	Parameter (g/100g)	Nutrient Content of Mushroom Powder
1	Moisture	10.30
2	Crude Protein	22.00
3	Crude Fat	1.80
4	Carbohydrate	42.70
5	Crude Fiber	15.70
6	Total Ash	3.60

Table-2: Nutritive Value of Fortified Mushroom Powder (Per 100 G)

S.No.	Parameters	Unit	Results
1	Protein	g/100 g	26.18
2	Total Carbohydrate	g/100 g	68.40
3	Dietary Fiber	g/100 g	1.26
4	Calcium	mg/100 g	289.52
5	Iron	mg/100 g	22.26
6	Zinc	mg/kg	674.67
7	Magnesium	mg/100 g	392.58

Organoleptic Evaluation Fortified Mushroom Powder

Table-3: Organoleptic Evaluation of Fortified Mushroom Powder

S. No	Parameter	Control (Whey Protein) T0	T1 (10%)	T2 (15%)	T3 (20%)	T4 (25%)	T5 (30%)
1	Appearance	7.5	6.7	5.5	6.4	7.8	7.6
2	Colour	7.5	7.0	5.7	6.5	7.8	7.4
3	Texture	7.5	7.1	5.9	6.4	7.7	7.5
4	Mouthfeel	7.8	6.7	6.3	6.6	7.9	7.3
5	Aftertaste	7.5	6.2	5.9	6.7	7.7	7.5
6	Overall Acceptability	7.7	6.8	5.8	6.6	7.8	7.7

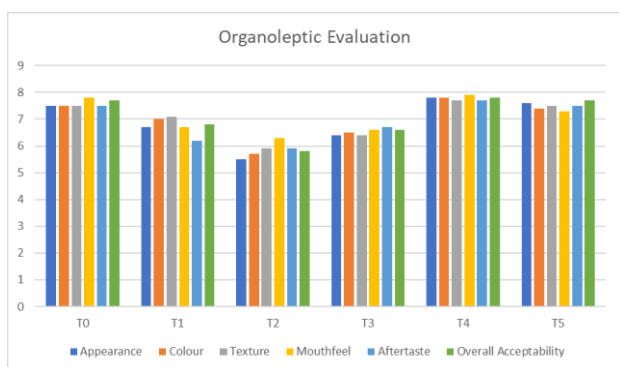


Fig. 9: Organoleptic Evaluation

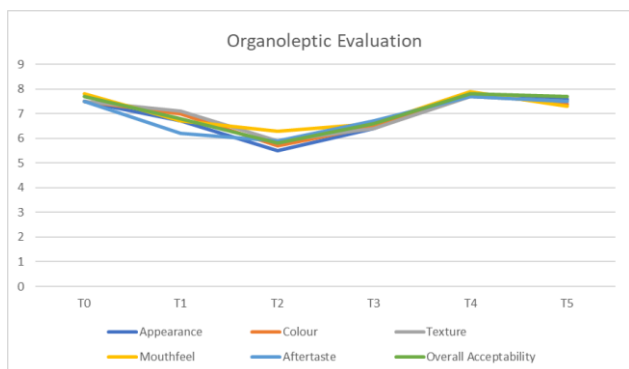


Fig. 10: Organoleptic Evaluation

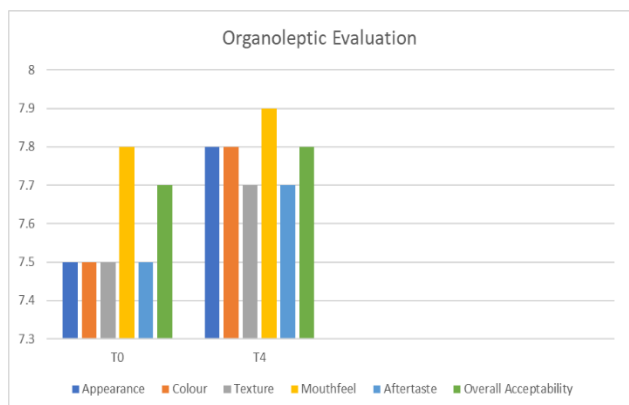


Fig. 11. Comparison Between Sample T0 and T4

G. Sensory Evaluation

An analysis was conducted on sensory data collected from 35 semi-trained panellists, comprising 10 men, 20 women, and 5 students. They used a 9-point hedonic rating scale to evaluate a developed product. The panellists assessed five treatments (T1, T2, T3, T4, and T5), with T0 serving as the control. The compositions of T1 through T5 are detailed in Table 3. The parameters used to record the panelists' responses included appearance, colour, texture, sweetness, mouthfeel, aftertaste, and overall acceptability.

- i. *Appearance*: The mean sensory scores for appearance for the fortified mushroom protein powder formulations T0, T1, T2, T3, T4, and T5 were recorded as 7.5, 6.7, 5.5, 6.4, 7.8, and 7.6, respectively.
- ii. *Colour*: The mean sensory scores for colour for the fortified mushroom protein powder formulations T0, T1, T2, T3, T4, and T5 were 7.5, 7.0, 5.7, 6.5, 7.8, and 7.4, respectively.
- iii. *Texture*: The mean sensory scores for texture for the formulations T0, T1, T2, T3, T4, and T5 were 7.5, 7.1, 5.9, 6.4, 7.7, and 7.5, respectively.
- iv. *Sweetness*: The mean sensory scores for sweetness for the formulations T0, T1, T2, T3, T4, and T5 were 7.8, 7.2, 6.9, 7.4, 8.0, and 7.5, respectively.
- v. *Mouthfeel*: The mean sensory scores for mouthfeel for the formulations T0, T1, T2, T3, T4, and T5 were 7.8, 6.7, 6.3, 6.6, 7.9, and 7.3, respectively.
- vi. *Aftertaste*: The mean sensory scores for aftertaste for the formulations T0, T1, T2, T3, T4, and T5 were 7.5, 6.2, 5.9, 6.7, 7.7, and 7.5, respectively.
- vii. *Overall Acceptability*: The mean sensory scores for overall acceptability for the formulations T0, T1, T2, T3, T4, and T5 were 7.7, 6.8, 5.8, 6.6, 7.8, and 7.7, respectively.

III. RESULTS AND DISCUSSION

The nutritive value of developed mushroom powder is crucial not only for determining its shelf life and nutritional quality but also for its suitability in creating specialized foods for specific purposes (Manzi, P.S., Marconi, A. A., Pizzoferrato, L. (2004) [10][13][14][15]. The sample (Mushroom Powder) underwent proximate analysis, and the results are presented in Table 2. Moisture content in flour significantly impacts its shelf stability, with lower moisture levels contributing to better storage stability. The table indicates that the moisture content of mushroom powder was 10.30%, similar to that of most cereals and millets. The average crude protein content in mushroom powder was 26.00%. The crude fat content averaged at 1.80%, indicating a low-fat content that is beneficial for heart health, as it is free from cholesterol. It was reported that a carbohydrate content of 68.4 % on a dry weight basis, was slightly higher than the tabulated data. Mushroom powder also showed an average dietary fiber content of 1.26%, highlighting its richness in high-quality fiber. The total ash content averaged 3.60%, indicating the presence of essential minerals. Overall, the mushroom powder is noted for its high protein, low fat, low calorie, high carbohydrate, high fiber content, and absence of cholesterol, making it a valuable dietary option.

IV. CONCLUSION

This study indicates that mushroom powder can be easily prepared under optimized process conditions. Organoleptic acceptability of mushroom fortified revealed that 25% fortification of mushroom powder in was liked extremely and also nutritious. Mushroom-fortified products are suggested for Children of growing age, pregnant and lactating women, and old persons who require a high-protein diet.

DECLARATION STATEMENT

After aggregating input from all authors, I must verify the accuracy of the following information as the article's author.

- **Conflicts of Interest/Competing Interests:** Based on my understanding, this article has no conflicts of interest.
- **Funding Support:** This article has not been sponsored or funded by any organization or agency. The independence of this research is a crucial factor in affirming its impartiality, as it has been conducted without any external sway.
- **Ethical Approval and Consent to Participate:** The data provided in this article is exempt from the requirement for ethical approval or participant consent.
- **Data Access Statement and Material Availability:** The adequate resources of this article are publicly accessible.
- **Authors Contributions:** The authorship of this article is contributed equally to all participating individuals.

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Prof. Kajal Verma, Associate Professor, is a highly seasoned and conscientious faculty member in Food Technology with over 12 years of experience in professional standards for safe food handling and distribution. She holds an MTech degree in Food

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Dr. Prity Pant, Professor, holds a Ph.D. in Dairy Technology with over 20 years of experience in food safety and agricultural education. She serves as a Professor of Practice at William Carey University and Director at the International Institute for Technical Teachers. Certified as a Lead Auditor in FSSC 22000 and HACCP, she has trained students and professionals across multiple institutions. Dr. Pant is also an FSSAI-empowered trainer and has published extensively in national and international journals. Her expertise spans food technology, safety management, and entrepreneurship in the agriculture sector.



The Dynamic Role of Ultrasonic Treatment Technology in Flax Seed for the Development of Fortified Mushroom Powder to Boost Nutritional Benefits in Drink Development



Dr. R. C. Mishra, Scientist, The Vice-Chancellor of Mahakaushal University, Dr. R. C. Mishra, holds a Ph.D. in Botany from the University of Allahabad and has over 18 years of experience in academic and research roles. He has served as a professor, dean, and head of the department at Swami Vivekanand University, with contributions to research in biopesticides and mycorrhizal fungi. He has authored several publications, led academic collaborations, and acted as an expert in various educational panels



Mr. Syed S. Abbas Director, is the Director of the Institute of Good Manufacturing Practices India (IGMPI). With extensive experience in pharmaceutical quality management, food safety, and regulatory affairs, he has been instrumental in shaping IGMP into a leading institution that offers industry-recognized certifications and training programs. Mr. Abbas focuses on advancing best practices in Good Manufacturing Practices (GMP) and compliance within industries such as pharmaceuticals, food, and healthcare. His leadership has positioned IGMP as a critical player in professional upskilling and regulatory education in India and internationally.

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