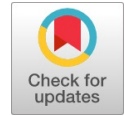


Boosting Nutritional Benefits: Ultrasonic Treatment of Flax and Pumpkin Seeds for Developing Fortified Mushroom Sprinkle Powder

Kajal Verma, Prity Pant, R C Mishra



Abstract: This study evaluated the physicochemical properties and sensory acceptability of fortified mushroom sprinkle powder formulated with blends of *Agaricus bisporus*, ground chia powder, ultrasonic-treated flaxseed powder, and ground pumpkin seeds. Standard analytical methods and Pearson's correlations were employed to analyse blends with varying mushroom flour concentrations (10%, 15%, 20%, 25%, and 30%). Results indicated that increasing mushroom content enhanced protein, ash, fibre, zinc, and iron levels in both composite flours and fortified smoothies. Additionally, *in vitro* protein digestibility of the smoothies improved with higher mushroom incorporation. The inclusion of *Pleurotus ostreatus* flour increased pH and decreased total titratable acidity (TTA), while *A. bisporus* flour exhibited the reverse effect. Elevated mushroom content also led to reductions in fat, carbohydrates, energy, and viscosity in the fortified flours and smoothies. Positive linear effects were observed for functional properties such as foaming capacity, foam stability, fat absorption, water retention, water absorption, solubility index, and swelling capacity, whereas compact density, bulk density, and syneresis decreased linearly. Gelation capacity, emulsifying activity, and emulsion stability were unaffected by *P. ostreatus* but showed slight decreases with *A. bisporus*. This study underscores the potential of mushroom flours as a valuable food fortification ingredient, offering enhanced nutritional profiles and various health-promoting benefits (R.M. Ishara Jackson1, Daniel N. Sila2, Glaston M. Kenji) (2018) [10].

Keyword: *Bisporus*, Ground Chia Powder, Ultrasonic-Treated Flaxseed Powder, Ground Pumpkin Seeds.

I. INTRODUCTION

The global population continues to grow, intensifying hunger and malnutrition in many regions. This issue is compounded by the declining nutritional value of staple foods due to nutrient-depleted soils. In developing countries, widespread malnutrition and a growing protein gap have spurred the search for alternative, sustainable protein sources.

With the production of pulses and cereals failing to meet the rising demand, the focus has shifted to nutrient-dense foods that promote health and strengthen disease immunity (Longvah, T.; Deosthale, Y.G., 1998) [4]. The Food and Agriculture Organization (FAO) has identified edible mushrooms as an excellent food source for protein nutrition, particularly for developing countries reliant on cereal-based diets. Rich in proteins, carbohydrates, vitamins, fibers, and essential minerals, commercially grown mushrooms are both safe and nutritious for human consumption.

Mushrooms, also known as toadstools, are the fleshy, spore-bearing fruiting bodies of fungi. They typically grow above ground on soil or their food substrate. The most commonly cultivated and consumed mushroom is the white button mushroom (*Agaricus bisporus*), often considered the standard reference for the term "mushroom." These fungi (classified as Basidiomycota, Agaricomycetes) are characterized by a stem (stipe), a cap (pileus), and gills (lamellae) beneath the cap, which produce microscopic spores that aid in dispersion. During peak harvest seasons, market oversaturation often forces growers into distress sales, resulting in significant losses from unsold mushrooms. To address this issue, it is crucial to develop methods for processing surplus mushrooms into value-added products. Such measures would not only prevent losses but also enhance growers' income through improved marketing and value addition. Implementing effective post-harvest technologies to create innovative mushroom-based products can help manage market fluctuations and maximize economic returns.

Mushroom protein, which ranks between vegetable and animal proteins in quality, serves as an essential supplementary protein source in vegetarian diets. Traditionally consumed cooked, raw, or as garnishes, mushrooms have a long history of use in Oriental medicine. However, their role as "health potentiators" and immune system enhancers has only recently gained recognition. Mushrooms are now widely acknowledged as functional foods with significant potential in pharmaceutical and nutraceutical development. They are valued for their antioxidant, antitumor, and antimicrobial properties, attributed to active components such as polysaccharides (β -glucans), dietary fibers, oligosaccharides, triterpenoids, peptides, glycoproteins, proteins, alcohols, phenols, and minerals like zinc, copper, iodine, iron, calcium, phosphorus, potassium, and selenium. Additionally, they contain essential vitamins and amino acids, making them a critical resource for advancing health and nutrition.

In recent years, rising affluence in developing countries has led to a notable increase in mushroom consumption,

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driven by their exceptional nutritional value (Bano, Z.; Rajarathnam, S., 2021) [6]. Mushrooms are low-calorie foods, rich in protein, fibre, vitamins, and minerals, while being low in carbohydrates, fat, and calories (Mattila, P.; Konko, K.; Euvola, M.; Pihlava, J.; Astola, J.; Vahteristo, L., 2001) [5]. Table 1 highlights the nutritional composition of mushroom powder. Widely incorporated into global cuisines, particularly in Chinese, Korean, European, and Japanese dishes, mushrooms are predominantly cultivated on farms, with *Agaricus bisporus* being the most commonly consumed species. Varieties such as white, crimini, and Portobello mushrooms, along with other cultivated types like shiitake, maitake (hen-of-the-woods), oyster, and enoki mushrooms, are readily available in grocery stores.

The growing interest in mushroom cultivation, especially in developing nations, has transformed it into a viable economic activity for small-scale farmers. Mushrooms and their extracts are also being explored for their potential therapeutic properties, including cardiovascular health benefits (Guillamón, E.; García-Lafuente, A.; Lozano, 2010) [3]. Research on specific mushroom components, such as polysaccharides, glycoproteins, and proteoglycans, suggests they may help modulate the immune system and inhibit tumour growth. Preliminary studies have also demonstrated antiviral, antibacterial, antiparasitic, anti-inflammatory, and antidiabetic potential in certain mushroom isolates. Historically valued for their medicinal properties, particularly in traditional Chinese medicine, mushrooms have been the subject of modern medical research since the 1960s, with most studies focusing on mushroom extracts rather than whole mushrooms (Stamets, P., 2005) [7].

The primary economic significance of mushrooms lies in their role as a food source for human consumption. Oyster mushrooms, in particular, are rich in vitamin C and B-complex vitamins, with a protein content ranging from 1.6% to 2.5% [11]. They also provide essential mineral salts required by the human body [12]. Remarkably, the niacin content in oyster mushrooms is approximately ten times higher than that found in most other vegetables, underscoring their nutritional superiority and potential health benefits [13].

Oyster mushrooms are a rich source of folic acid, which is beneficial in treating anaemia. Their low sodium-to-potassium ratio, combined with minimal starch, fat, and caloric content, makes them ideal for individuals managing hypertension, obesity, and diabetes. Additionally, their alkaline ash content and high fibre levels help alleviate hyperacidity, constipation, and high cholesterol. As one of the few vegan sources of vitamin D and conjugated linoleic acid, mushrooms also provide antioxidant properties through compounds such as ergothioneine. Oyster mushrooms thrive in moderate temperatures (20–30°C) and humidity levels of 55–70% for 6–8 months a year. With added humidity, they can even be cultivated during summer, particularly in hilly areas above 900 meters, where the prime growing season spans March/April to September/October. In lower regions, the growing season typically shifts to September/October through March/April.

The combination of mushrooms with chia seeds, flax seeds, and pumpkin seeds offers a powerful nutritional synergy. Fortified mushroom smoothies can be developed by

integrating these ingredients to provide slow-releasing carbohydrates, rich antioxidant content, and enhanced health benefits, making them especially suitable for individuals with celiac disease.

Mushrooms are highly regarded as nutraceutical foods due to their substantial functional and nutritional properties (Chang, S.T.; Miles, P.G., 2008) [1]. They are celebrated not only for their economic value but also for their sensory appeal and medicinal benefits. The distinction between edible and medicinal mushrooms is often blurred, as many edible varieties also possess therapeutic potential. The most commonly cultivated mushroom species include *Agaricus bisporus*, *Flammulina velutipes*, *Lentinus edodes*, and various *Pleurotus* species.

Edible mushrooms are noted for their high crude protein content, though this varies by species and developmental stage. While their levels of free amino acids are relatively low (7.14–12.3 mg g⁻¹ dry weight), these compounds significantly contribute to their flavour profiles (Maga, 1981). However, mushrooms are naturally low in sulphur-containing amino acids such as methionine and cysteine, while being relatively rich in threonine and valine. This unique amino acid composition adds to their value as a functional and nutritionally significant food source.

Cultivated mushrooms are an excellent source of essential vitamins, including folates, niacin, and riboflavin, with particularly high levels of vitamin B2 compared to most vegetables, making them a valuable addition to a balanced diet. The folate in mushrooms is primarily in the bioavailable form of folic acid. They also contain small amounts of vitamin B1, vitamin C, and trace amounts of vitamin B12. While their total soluble sugar content is low, mushrooms are rich in oligosaccharides.

The carbohydrate content in edible mushrooms varies significantly across species, ranging from 35% to 70% by dry weight. Their fat content is relatively low, typically between 2% and 8%, with polyunsaturated fatty acids making up more than 75% of the total fatty acid profile. Saturated fatty acids are present in lower amounts, with palmitic acid being the predominant type. This unique nutritional composition highlights the dietary and health benefits of cultivated mushrooms. (Ergönül, P.G.; Akata, I.; Kalyoncu, F.; Ergönül) (2013) [2].

II. MATERIAL AND METHOD

The current research was conducted at the Department of Food Safety and Quality Laboratory, IGMPI, C-6, Qutub Institutional Area, near the Old JNU Campus, New Delhi.

Procurement of Raw Materials and Product Development: Fresh oyster mushrooms were sourced from the local market. Mushroom powder was prepared using the oven-drying method, as illustrated in Flow Chart 1. Fortified mushroom powder was then developed by replacing whey protein with mushroom powder. Additionally, powdered forms of grounded flax seeds, chia seeds, and pumpkin seeds were incorporated in significant proportions to enhance the nutritional value.

Ultrasound Technology:
Ultrasound technology operates using mechanical



waves with frequencies beyond the range of human hearing (above 16 kHz). These ultrasonic waves are categorized into low-intensity and high-intensity groups based on their frequency. Low-intensity ultrasound, also referred to as non-destructive or high-frequency ultrasound, employs high-frequency waves (100 kHz–1 MHz) and low power (<1 W/cm²). This type of ultrasound is used in analytical methods to assess the physical and chemical properties of food products, including parameters like acidity, ripeness, firmness, and sugar content. (Mohammed & Alhajhoj, 2019) [9].

High-intensity ultrasound, also referred to as low-frequency or power ultrasound, utilizes high power levels (10–1000 W/cm²) and low frequencies (16–100 kHz) to improve food quality. This technique induces physical and chemical changes through its powerful, low-frequency ultrasonic waves (Mohammed & Alhajhoj, 2019) [9]. Additionally, ultrasound has proven to be an effective cleaning method. During ultrasonic cleaning, foulant removal occurs either through chemical interactions between the foulants and radicals generated by ultrasonic treatment in the liquid or via the mechanical effects of ultrasound in the liquid medium. The most commonly used systems for this purpose are the ultrasonic bath and the probe system of sonochemistry apparatuses, which are readily available to chemists. Ultrasound offers several advantages, including improved productivity, higher yields, greater selectivity, faster processing times, enhanced quality, minimal chemical or physical damage, and environmental sustainability.

Cavitation, a phenomenon that occurs when liquid pressure is significantly reduced, causes the liquid to burst and form vapor bubbles. Ultrasonication operates based on the cavitation theory. Bubbles form in a liquid exposed to ultrasonic waves through the following mechanisms:

High-intensity, low-frequency ultrasound (20 kHz) rapidly generates cavitation nuclei due to inertial effects. Low-intensity, high-frequency ultrasound (above 50 kHz) also forms cavities, albeit at a slower expansion rate, as the cavity's surface area increases marginally with compression compared to expansion.

When a cavity grows beyond a certain size, it can no longer absorb sufficient energy from the sound field to sustain itself; this critical size depends on the ultrasound frequency. Cavitation created by ultrasonic waves allows the solvent to penetrate cells effectively, initiating the extraction process. This process also generates macroturbulence, high-velocity particle collisions, and movement in cell walls or the microporous matrix. Directing ultrasonic jets at solid surfaces enhances the contact area between liquids and solids, improving the efficiency of bioactive component extraction.

In this study, flax seeds and pumpkin seeds underwent ultrasonic treatment before being ground into flour for developing fortified mushroom powder. Ultrasound, depending on its intensity, is applicable to a wide range of processes, including enzyme activation or deactivation, mixing and homogenization, emulsification, dispersion, preservation, stabilization, dissolution, crystallization, hydrogenation, meat tenderization, ripening, aging, and oxidation. It is also used as an auxiliary technique for solid-

liquid extraction, enhancing and accelerating 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 (labelled 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

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 fibre 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. 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 fibre and gums in the pre-treatment process.



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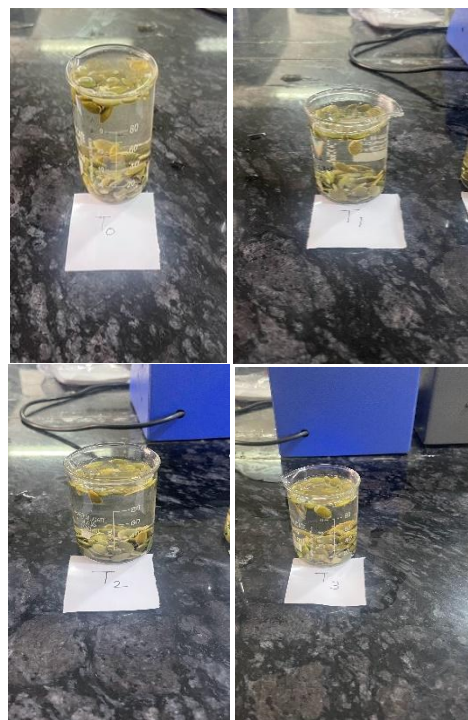
[Fig.1: Ultrasonic Machine]

B. Ultrasonic Treatment of Pumpkin Seeds

Pumpkin 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 (labelled T0, T1, T2, and T3). 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.



[Fig.2: Ultrasonic Machine]



[Fig.3: Pumpkin Samples for T0, T1, T2, and T3]

Pre-Treatment of Pumpkin Seeds: Pumpkin seeds were subjected to ultrasonic treatment in four trials, designated T0, T1, T2, and T3, 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 fibre 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. 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 pumpkin seeds. From the four trials T4 was found to have more soluble fibre and gums in the pre-treatment process.



[Fig.4: Pre-treatment Process of Pumpkin Seeds]



[Fig.5: After Pre-treatment Process of Pumpkin Seeds]

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.

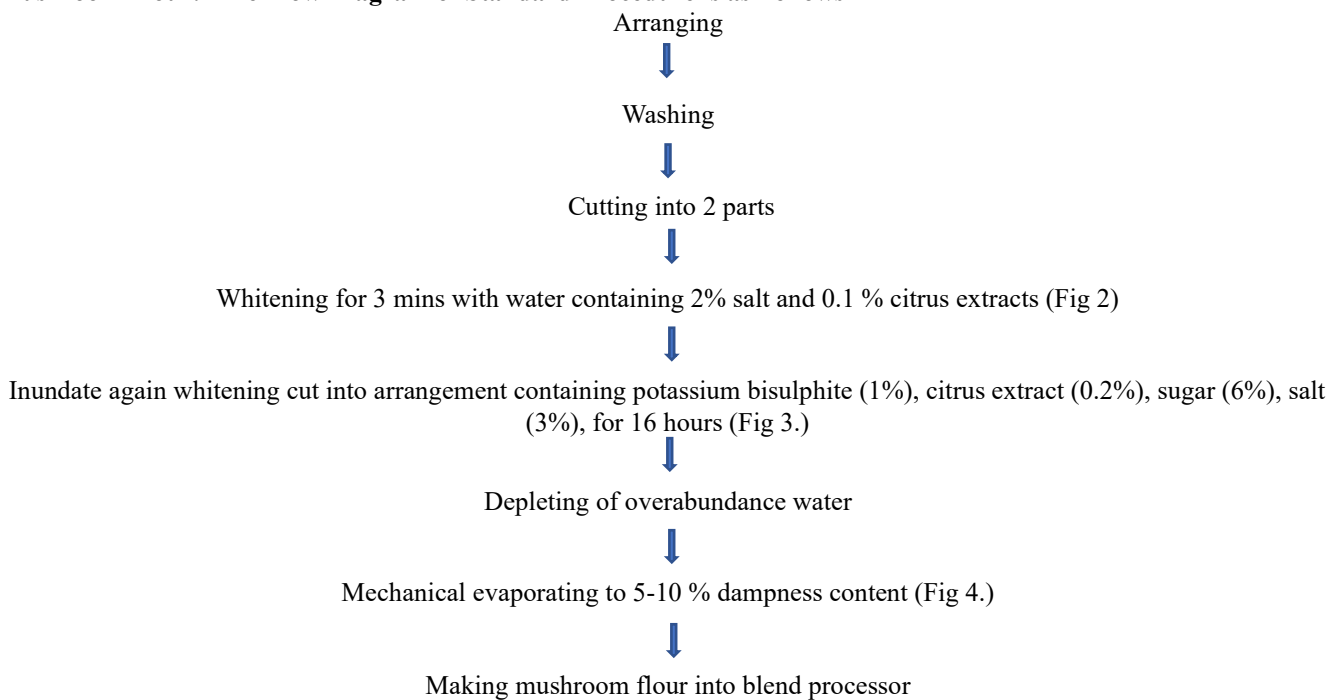
Score	Scale Grade
1	Disliked immensely
2	Disliked exceptionally
3	Disliked more than a little
4	Disliked a little
5	Neither liked nor disliked
6	Liked a little
7	Liked more than a little
8	Liked exceptionally
9	Liked immensely

Nutritive Value: The developed products were analysed for proximate principles, including moisture, crude protein, crude fat, carbohydrate, total ash, and crude fibre, using standardized procedures from AOAC (1980).

Statistical Analysis: Analysis of variance (ANOVA) was conducted to analyse 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.

- a) Salt
- b) Citrus extract
- c) Potassium bisulphite
- d) black pepper
- e) Oven
- f) Mixer Grinder
- g) flax seed powder
- h) Pumpkin seed powder
- i) Chia seed powder
- j) oregano
- k) mushroom powder.
- l) cumin powder

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



Flow Chart 1

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[Fig.6: Cutting of Mushroom]



[Fig.7: Dipping the Mushroom into the Saline Solution for 16 Hours]



[Fig.8: Dipping the Mushroom Into the Saline Solution for 16 Hours]



[Fig.9: Drying the Mushroom Into Oven for 6 Hours at 50°C]



[Fig.10: Dried Mushroom Flour]

Mixture of Mushroom Flour Along with Chia Seeds, Flax Seeds and Pumpkin Seeds in the Blend form]



[Fig.11: Fortified Mushroom Product of Mushrooms Flour]

Mushroom Fortified Sprinklers – 25 gm of Mushroom Fortified sprinklers can be prepared with mushroom powder (2.5 gm), Flax seed Powder (2.5 gm), Chia seed powder (2.5 gm), Pumpkin seeds (2.5 gm), black pepper (2 gm), salt (2 gm), oregano (5 gm), cumin powder (5 gm). This Mushroom fortified sprinkler can be used for enhancing the taste of salad, and can be used in any curry and for sprinkling on any dishes. This provides value addition for daily diet for adults, senior citizens, and youngsters also who are very fond of Chinese dishes.

Table 1: Nutritive Value of Developed Mushroom Powder

S. No	Parameter (g/100g)	Nutrient Content of Mushroom Powder
1	Moisture	9.30
2	Crude Protein	20.00
3	Crude Fat	1.50
4	Carbohydrate	40.70
5	Crude Fiber	1.20
6	Total Ash	3.40

Table 2: Nutritive Value of Fortified Mushroom Powder (per 100 g)

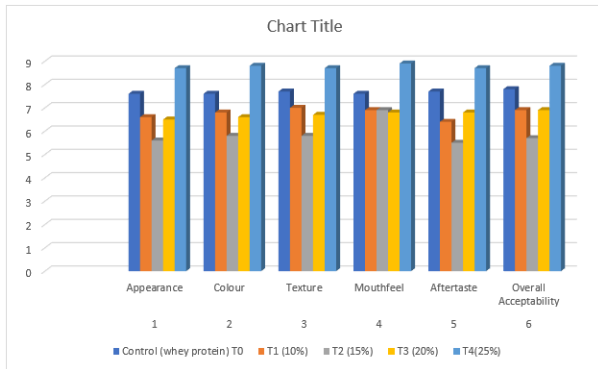
S. No.	Parameters	Unit	Results
1	Protein	g/100 g	27.18
2	Total Carbohydrate	g/100 g	67.40
3	Dietary Fibre	g/100 g	1.14
4	Calcium	mg/100 g	284.52
5	Iron	mg/100 g	20.16
6	Zinc	mg/kg	670.62
7	Magnesium	mg/100 g	371.41

Organoleptic Evaluation Fortified Mushroom Powder

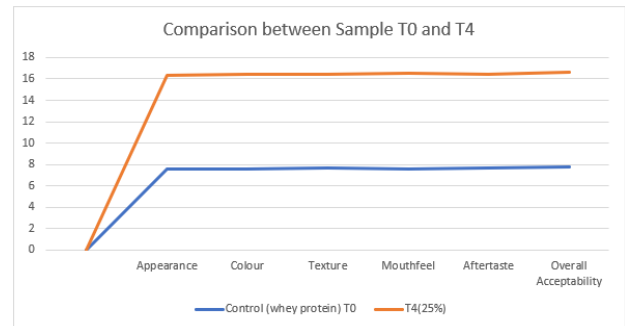
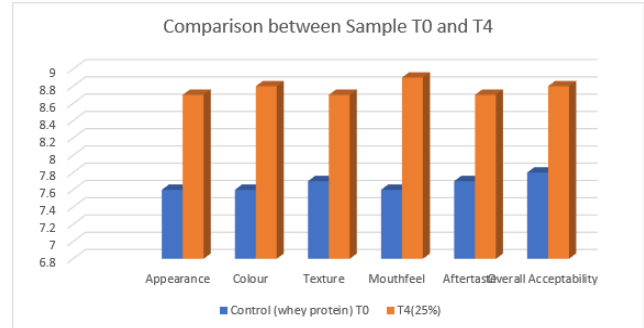


Table 3: Organoleptic Evaluation of Fortified Mushroom Powder

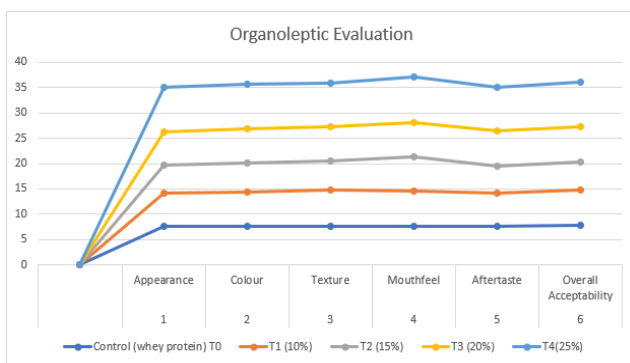
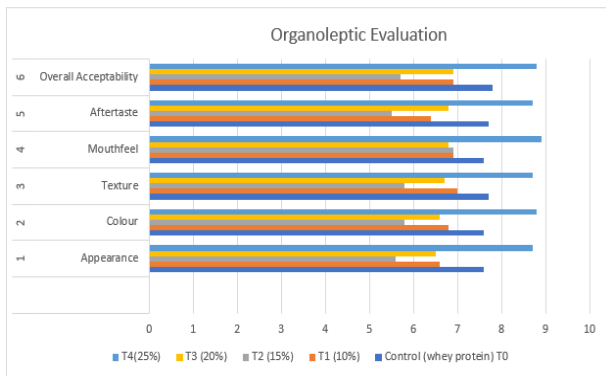
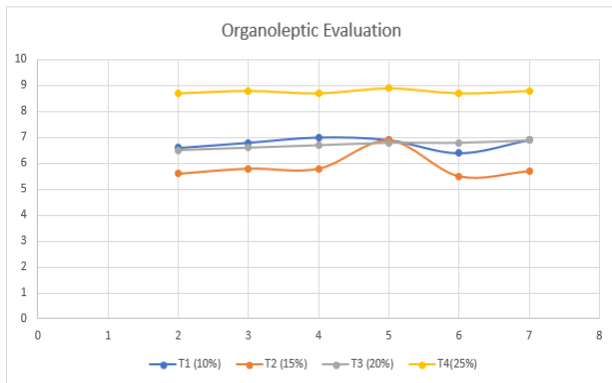
S.no	Parameter	Control (Whey Protein) T0	T1 (10%)	T2 (15%)	T3 (20%)	T4 (25%)
1	Appearance	7.6	6.6	5.6	6.5	8.7
2	Colour	7.6	6.8	5.8	6.6	8.8
3	Texture	7.7	7.0	5.8	6.7	8.7
4	Mouthfeel	7.6	6.9	6.9	6.8	8.9
5	Aftertaste	7.7	6.4	5.5	6.8	8.7
6	Overall Acceptability	7.8	6.9	5.7	6.9	8.8



[Fig.12: Organoleptic Evaluation]



[Fig.14: Comparison Between Sample T0 and T4]



[Fig.13: Organoleptic Evaluation]

C. Sensory Evaluation

An analysis was conducted on sensory data collected from 30 semi-trained panellists, comprising 10 men, 15 women, and 5 students. They used a 9-point hedonic rating scale to evaluate a developed product. The panellists assessed five treatments (T0, T1, T2, T3, and T4), with T0 serving as the control. The compositions of T0 through T4 are detailed in Fig 15. The parameters used to record the panellists' responses included appearance, colour, texture, sweetness, mouthfeel, aftertaste, and overall acceptability.

Appearance: The mean sensory scores for appearance for the fortified mushroom protein powder formulations T0, T1, T2, T3, and T4 were recorded as 7.6, 6.6, 5.6, 6.5, and 8.7 respectively.

Colour: The mean sensory scores for colour for the fortified mushroom protein powder formulations T0, T1, T2, T3, and T4 were 7.6, 6.8, 5.8, 6.6, and 8.8 respectively.

Texture: The mean sensory scores for texture for the formulations T0, T1, T2, T3, and T4 were 7.7, 7.0, 5.8, 6.7, and 8.7 respectively.

Mouthfeel: The mean sensory scores for mouthfeel for the formulations T0, T1, T2, T3, and T4, were 7.6, 6.9, 6.9, 6.8, and 8.9 respectively.

Aftertaste: The mean sensory scores for aftertaste for the formulations T0, T1, T2, T3, and T4 were 7.7, 6.4, 5.5, 6.8, and 8.7 respectively.



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Overall Acceptability: The mean sensory scores for overall acceptability for the formulations T0, T1, T2, T3, and T4 were 7.8, 6.9, 5.7, 6.9, and 8.8 respectively.

III. RESULTS AND DISCUSSION

The nutritional profile of the developed mushroom powder is essential not only for assessing its shelf life and quality but also for its application in creating specialized food products for targeted purposes (Manzi, P.S., Marconi, A.A., Pizzoferrato, L., 2004) [8]. Proximate analysis was conducted on the mushroom powder, and the findings are summarized in Table 2.

Moisture content plays a critical role in shelf stability, with lower levels enhancing storage life. The moisture content of the mushroom powder was recorded at 9.30%, comparable to that of most cereals and millets. The crude protein content averaged 20.00%, reflecting its high protein value. The crude fat content was low, averaging 1.50%, making it heart-healthy due to its cholesterol-free nature. The carbohydrate content was 40.70% on a dry weight basis, slightly exceeding previously reported data. Additionally, the mushroom powder contained an average dietary fiber content of 1.20%, emphasizing its high-quality fibre richness. The total ash content averaged 3.40%, indicating the presence of vital minerals.

Overall, the mushroom powder stands out for its high protein, low fat, low calorie, high carbohydrate, and high fibre content, along with its cholesterol-free composition, making it an excellent dietary choice.

IV. CONCLUSION

This study demonstrates that mushroom powder can be efficiently prepared under optimized processing conditions. The sensory evaluation of mushroom-fortified products revealed that 25% fortification with mushroom powder was highly favoured and recognized as nutritious. Mushroom-fortified products are recommended for children in their growth stages, pregnant and lactating women, and elderly individuals who require a protein-rich 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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