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Phytochemical and proximate analysis of protein based bar prepared by oyster mushroom

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Abstract
Oyster mushrooms (Pleurotus ostreatus) have emerged as a versatile and nutritionally valuable fungi with significant potential in the food industry. Native to temperate and subtropical regions, these mushrooms have been cultivated globally due to their ease of growth, high yield, and adaptability to diverse agricultural by-products as substrates. Their production involves environmentally sustainable practices, utilizing lignocellulosic waste materials such as straw, sawdust, and husks, thereby contributing to waste reduction and promoting circular agricultural systems. Pleurotus ostreatus is known for its remarkable nutritional profile, including highquality proteins rich in essential amino acids, dietary fibers, vitamins, and bioactive compounds like β-glucans and polyphenols, which are associated with numerous health benefits such as immune system support, cholesterol regulation, and antioxidant effects.This review highlights the innovative application of oyster mushrooms in the formulation of protein bars, leveraging their functional properties such as binding, texture enhancement, and prolonged shelf stability. The incorporation of these mushrooms into protein bars not only enriches their nutritional value but also aligns with consumer demands for plant-based, nutrient-dense food products. Additionally, the potential of Pleurotus ostreatus in addressing sustainability challenges and catering to modern dietary preferences underscores its relevance in functional food development. By tapping into the nutritional and functional properties of Pleurotus ostreatus, this paper aims to contribute to the advancement of innovative and sustainable food products that align with contemporary health and environmental goals.

Keywords: Oyster Mushroom, Protein Bar, Phytochemical and Proximate Analysis

Introduction
Oyster mushrooms (*Pleurotus ostreatus*) are gaining attention as a promising ingredient in the development of protein bars, in line with the rising demand for plant-based and sustainable food options. As consumers become more aware of the environmental impact of their diets, there is growing interest in alternative protein sources that are nutritious, eco-friendly, and affordable. Oyster mushrooms, being low-impact crops, are well-positioned to meet this demand by offering high-quality protein, essential vitamins, and minerals (A. Masnar *et al*., 2024) [1]. They are particularly suitable for inclusion in functional foods due to their nutritional value and potential health benefits. Studies have demonstrated their capacity to help manage chronic diseases such as cardiovascular disease, diabetes, cancer, and neurological disorders, largely due to their bioactive compounds such as antioxidants and unsaturated fatty acids. The incorporation of oyster mushrooms into protein bar formulations not only boosts their nutritional content but may also enhance their therapeutic potential. Oyster mushrooms are rich in protein, fiber, and essential micronutrients. Their cultivation is relatively simple and sustainable, using cellulose-rich agricultural waste as a substrate. Research has reported protein content as high as 28.45 mg/g in these mushrooms, along with significant amounts of essential amino acids, vitamins (A, B1, B6, E), and minerals such as calcium, phosphorus, and potassium (Fatimah Buba *et al*., 2022) [2]. These attributes make oyster mushrooms ideal for formulating health-oriented foods like protein bars, especially for athletes and health-conscious consumers. Moreover, oyster mushroom protein has been shown to possess antioxidant, anti-inflammatory, and antibacterial properties, enhancing the functional appeal of food products. Such bioactive effects could add value to protein bars by supporting immunity, reducing inflammation, and offering protection against oxidative stress (Vrinda Bansal *et al*., 2020) [3].

Mushrooms like *Pleurotus ostreatus* are increasingly recognized as important dietary components due to their health-promoting properties. Their popularity in regions like Asia and Europe stems from both their taste and nutritional benefits. In Bangladesh and similar climates, they are widely cultivated due to favorable conditions and their potential to address nutritional deficiencies.

Oyster mushrooms contain 19–35% protein on a dry weight basis, outperforming common staples such as rice (7.3%) and wheat (13.2%). They also offer 4% fat, primarily in the form of beneficial unsaturated fatty acids. A 100g serving provides about 2.5g of dietary fiber and is rich in vitamins C, D, and B-complex. Importantly, they are one of the few plant-based sources of cobalt, which plays a role in vitamin B12 synthesis. They are also abundant in selenium, a trace element crucial for protecting cells from oxidative damage (Bhattacharjya D.K. *et al*., 2015) [4].

Oyster mushrooms provide a variety of health benefits that support their use in functional foods:

1. **Immune Support:** Contain beta-glucans that enhance immune response.
2. **Antioxidant Effects:** Rich in compounds like polyphenols and ergosterol that fight free radicals.
3. **Cardiovascular Health:** Help lower cholesterol and reduce heart disease risk.
4. **Anti-inflammatory Properties:** May help relieve conditions such as arthritis.

In conclusion, oyster mushrooms offer a compelling case as a primary ingredient in protein bars due to their nutritional density, bioactive properties, sustainability, and ease of cultivation. This study emphasizes evaluating oyster mushrooms' potential in protein bar formulations based on nutritional composition, functional benefits, and consumer appeal.

Material and Methods

The current study aimed to create and assess a mushroom-based protein bar with Pleurotus ostreatus (oyster mushroom) as the main ingredient. The study was conducted at the Food Science Analysis Laboratory, Department of Food and Nutrition, School of Home Science, Babasaheb Bhimrao Ambedkar University (BBAU), Lucknow, from July 2024 to May 2025. To meet the research aims, an experimental research design was used, which was divided into five critical phases. During the initial phase, the protein bar was created and standardized utilizing oyster mushrooms in different trial formulations to improve sensory and nutritional qualities. The second phase involves proximate analysis of the completed formulation to assess moisture, protein, fat, fiber, ash, and carbohydrate content using conventional AOAC (2016) techniques. The third phase focused on analyzing the product's functional and sensory qualities. Functional aspects such as texture and binding capability were evaluated, while organoleptic evaluation was performed by a semi-trained panel utilizing a 9-point hedonic scale to measure sensory characteristics such as taste, color, scent, texture, and overall attractiveness. To determine the product's economic feasibility, a full cost study was conducted in the fourth phase, which included raw material costs, processing, labor, and packaging. The fifth phase tested the shelf life of protein bars by storing them at various temperatures (25 ± 2°C and 4 ± 1°C) and monitoring sensory quality, microbial load, and physicochemical properties over a 60-day period.

Chemicals used

The nutritional and bioactive components of mushroom-based protein bars are usually evaluated using conventional laboratory reagents for phytochemical and proximate analysis. Proximate analysis uses sulfuric acid, sodium hydroxide, and boric acid for protein estimation using the Kjeldahl method; petroleum ether or hexane for fat extraction using the Soxhlet apparatus; and oven drying for moisture content. Phytochemical screening employs reagents such as Mayer's Wagner's and Dragendorff's for alkaloids, ferric chloride for tannins, Folin-Ciocalteu reagent for phenolics, and sodium hydroxide or lead acetate for flavonoids. These substances aid in the quantification of important nutrients as well as the identification of bioactive molecules that contribute to the protein bars' functional and therapeutic value.

Sample collection

Fresh oyster mushrooms, pumpkin seeds, peanuts, and almonds were obtained from local, recognized providers. The items were cleaned, roasted, or dried under regulated conditions, then ground or chopped as needed. Dry date powder was utilized as a natural sweetener, with guar gum powder acting as a binding agent. Vanilla essence was added to enhance the flavor.

Preparation of product

A mushroom-based protein bar was developed utilizing oyster mushroom powder along with nutrient-rich ingredients like almonds, peanuts, pumpkin seeds, dry date powder, and cocoa powder. The formulation aimed to make mushroom protein the primary protein source, enhancing flavor and nutritional quality through careful selection of other components. Guar gum powder served as a natural binder, while vanilla essence was added for improved aroma and taste. The development process involved basic kitchen and laboratory tools such as grinders, sieves, and mixing bowls. Variations of the bars were created by adjusting the amounts of dried oyster mushroom powder—5g (T1), 7.5g (T2), and 15g (T3)—while keeping other ingredients consistent. Ingredients were precisely weighed, and peanuts, pumpkin seeds, and almonds were pan-roasted at 55–60°C for 15 minutes for flavor enhancement and shelf stability. The dried mushrooms were also ground and lightly roasted. After cooling, the ingredients were ground into granules, mixed, and combined with a guar gum slurry for binding. The mixture was then molded into bars, frozen to set, and stored under frozen conditions to maintain texture, binding integrity, and nutritional value.

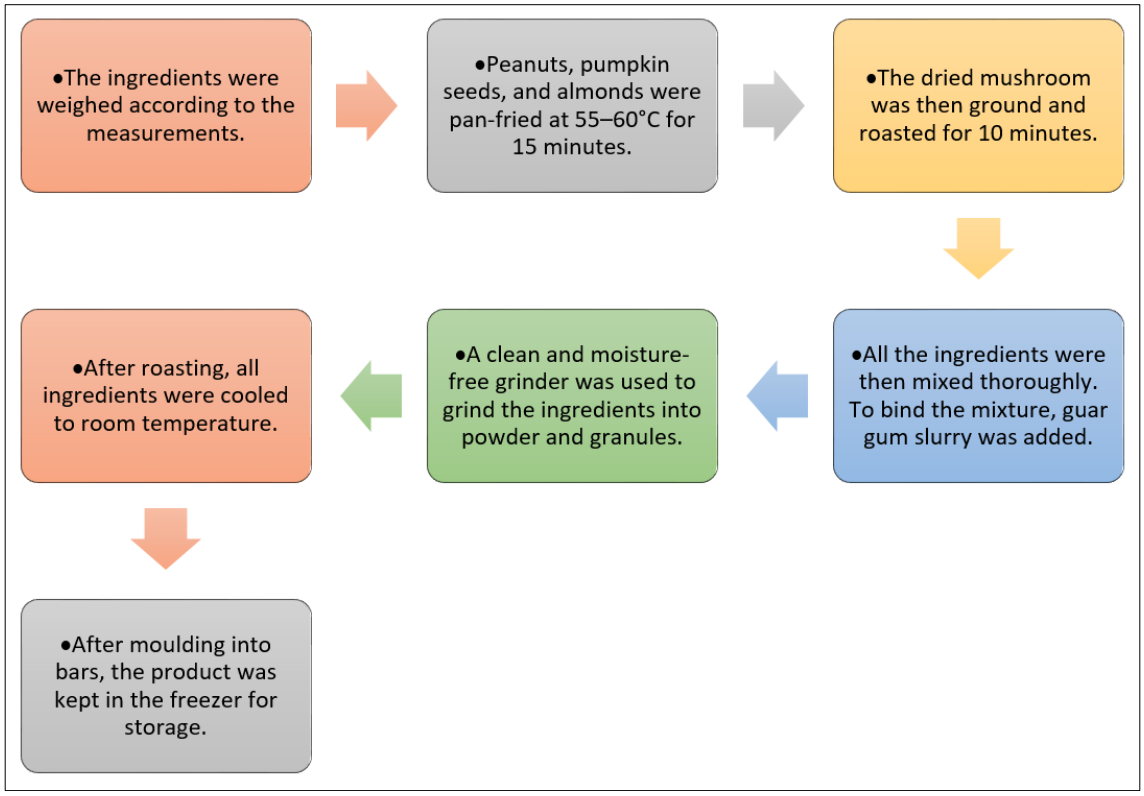

Fig 1: Flowchart of preparation of protein bar

Phytochemical Analysis and Proximate Analysis

The study of chemical substances called phytochemicals that plants produce to fend off environmental stressors is known as phytochemical analysis. These bioactive substances, which include terpenoids, alkaloids, and flavonoids, have anti-inflammatory and antioxidant qualities. Finding and measuring these substances is the main goal of food and nutraceutical research in order to evaluate the nutritional content of plant-based products. The analysis entails solvent extraction followed by methods such as GC-MS and HPLC. While quantitative assays evaluate elements like total phenolic content, qualitative screenings identify certain classes. Diets high in phytochemicals have been linked in studies to a decreased risk of chronic illnesses. In the end, phytochemical analysis is essential to pharmacology and food science for confirming health claims and guaranteeing product quality. In order to assess the nutritional makeup of protein bars and measure elements like moisture, ash, crude protein, crude fat, crude fiber, and carbs using AOAC methodologies, proximate analysis is crucial. For accurate results, studies have used established methodologies to test these components in a variety of protein bars, including those manufactured from wheat germ and lupine. For instance, mycoprotein-fortified bars were assessed for moisture (oven drying), crude protein, fat, fiber, and ash, and the difference between them was used to compute the amount of carbs. This approach was supported by additional studies on organic protein bars, and the results showed that the addition of fiber and protein significantly altered the bars' nutritional value. Using AOAC protocols consistently across fortification studies enables efficient nutritional profiling and comparison of various formulations.

Result and Discussion

Result: Phytochemical Analysis

Table 1: Results of phytochemical tests

S. No.	Phytochemical tests	Standard sample	T2 sample
1	Flavonoid	-ve	-ve
2	Steroid	- ve	- ve
3	Quinones	+ ve	+ ve
4	Tannins	+ ve	+ ve
5	Saponins	+ ve	-ve
6	Terpenoids	+ ve	-ve
7	Glycosides	-ve	-ve

Both parallels and differences can be seen when comparing the ingredients of the Standard Mixture and the Prepared Product supplement. There is uniformity in the presence of flavonoids, quinones, phenols, tannins, and glycosides in the formulations of the Standard Mixture and the Prepared Product. But the Standard combination contains steroids, whereas the Prepared product does not. The Standard Mixture and Prepared Product formulations, however, lack saponin. These results imply that the Standard Mixture and the Prepared Product have certain components in common, but they also differ significantly.

Proximate analysis

Table 2: Results of proximate analysis

S. No.	Parameter	Result (standard)	T2 sample
1	Moisture content	11.73%	14.35%
2	Ash content	2.44%	3.5%
3	Crude fat	10.53%	10.53%
4	Crude fibre	9.15%	10.66%
5	Protein content	21.3%	22.4%
6	Carbohydrate	35.3%	36.9%
7	Energy	485.3 kcal	471.7 kcal

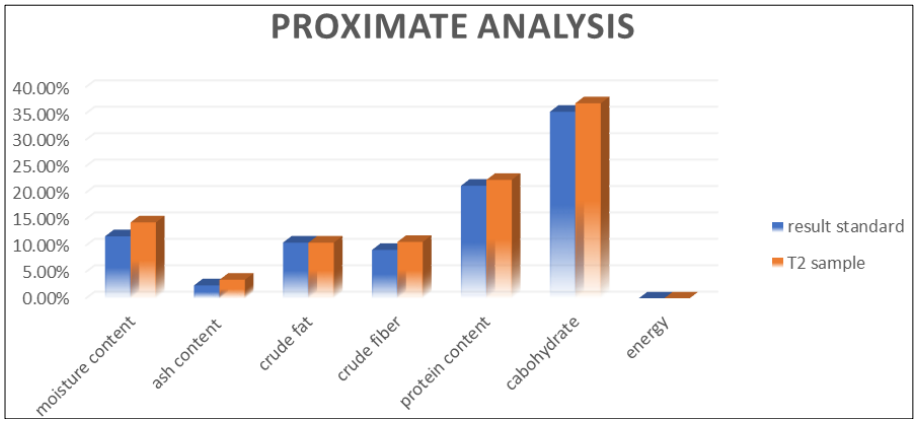

Fig: 2 Graph shows proximate analysis

The analysis of the prepared product revealed the following compositional data: Moisture content was measured at 11.73% for the standard sample and 14.35% for the T2 sample. Ash content was recorded at 2.44% for the standard and 3.5% for T2. The total crude fat content remained consistent at 10.53% in both the standard and T2 samples. Crude fiber content was noted to be 9.15% for the standard, while the T2 sample exhibited a higher level at 10.66%. The protein content increased from 21.3% in the standard to 22.4% in T2, indicating potential modifications in the T2 sample that enhanced its protein level. Carbohydrate content was 35.3% for the standard and slightly higher at 36.9% for T2. Lastly, total energy values were identified as 485.3 kcal for the standard and 471.7 kcal for the T2 sample.

Discussion

The viability of using edible mushrooms as a substitute protein source in functional food development was shown by the creation of a protein bar made with Pleurotus ostreatus powder. According to the proximate analysis, the addition of mushroom powder, especially in the T2 formulation, resulted in a significant rise in the content of crude fiber (10.66%) and protein (22.4%) as compared to the standard sample. These improvements show that P. ostreatus improves the protein density and dietary fiber profile of the bar while also making a nutritional and functional contribution. The presence of bioactive components such quinones and tannins, which are recognized for their antioxidant and health-promoting qualities, was verified by phytochemical analysis. The T2 sample's partial lack of terpenoids, steroids, and saponins points to compositional variations impacted by heat treatment and formulation. Furthermore, the mushroom component's mineral richness and hygroscopic nature may be the cause of the T2 formulation's increased moisture and ash concentrations, which could have an impact on the product's textural qualities and shelf lifespan. Overall, these findings emphasize oyster mushroom powder's potential as a functional ingredient that might improve the nutritional, biochemical, and sensory properties of protein-based snack compositions. The findings lend credence to its use in the development of sustainable, plant-based, nutrient-dense goods aimed at health-conscious customers.

Conclusion

The current study's findings support the significant potential of Pleurotus ostreatus (oyster mushroom) as a useful and sustainable ingredient in the formulation of protein-based food items. The use of oyster mushroom powder in protein bar formulations significantly improved the nutritional profile and added health-promoting bioactive substances. The results of both the proximate and phytochemical studies support the viability of using Pleurotus ostreatus to increase the nutritional quality, functional properties, and therapeutic value of ready-to-eat snack products.

The proximate analysis demonstrated significant improvements in key nutritional parameters in the T2 formulation (7.5 g mushroom powder) over the standard sample. The observed increase in protein content from 21.3% to 22.4% emphasizes oyster mushrooms' role as an effective plant-based protein source. Furthermore, the increase in crude fiber (from 9.15% to 10.66%) and ash content (from 2.44% to 3.5%) indicates the mushroom substrate's mineral richness and fibrous composition. Although the T2 sample had a slightly higher moisture level (14.35%), this was still within acceptable limits for nutritionally dense snack products and could be attributable to the hygroscopic properties of mushroom ingredients.

The presence of many bioactive chemicals, such as quinones and tannins, which are well known for their antibacterial and antioxidant qualities, was verified by phytochemical screening of the proposed formulation. The discovery of these chemicals lends weight to the idea that oyster mushroom-based dietary products may provide functional health benefits by lowering oxidative stress and boosting cellular protection. The partial lack of saponins, steroids,

and terpenoids in the T2 formulation could be due to thermal degradation during the roasting and processing stages; nonetheless, the survival of important bioactive elements emphasizes the functional importance of oyster mushroom inclusion. These findings are consistent with prior studies, indicating that P. ostreatus is an important source of phytochemicals with potential therapeutic applications.

The results also highlight the advantages of oyster mushroom farming for sustainability from an economic and environmental standpoint. Pleurotus ostreatus can be cultivated on a range of lignocellulosic agricultural leftovers, such as straw, husks, and sawdust, helping to reduce agro-waste and promote a circular agricultural economy. Compared to traditional protein sources, oyster mushroom farming requires fewer resources and has a reduced environmental impact, establishing it as an environmentally responsible choice for food innovation.

According to the experiment, protein bars with enhanced nutritional, functional, and sensory properties can be made with oyster mushroom powder. The improved protein enrichment, fiber content, and phytochemical availability of the revised T2 formulation demonstrated its suitability as a functional meal for consumers who are health-conscious. The use of mushrooms as nutrient-dense and sustainable ingredients in modern food systems is further supported by this study. Antioxidant efficacy, amino acid compositions, consumer acceptability across various demographics, and long-term storage viability should all be thoroughly assessed in future studies. Pleurotus ostreatus's successful integration into protein snack items is a possible path for the creation of plant-based functional meals that support sustainability and global health objectives.

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Conflict of Interest

There is no conflict of interest between the authors.

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