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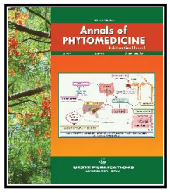

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Enrichment of proso millet cookies with oyster mushroom, *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. for enhanced nutritional and antioxidant properties

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Abstract

Food enrichment is a vital approach to improving our diets' nutritional value and fostering general well-being. Enhancing food products' nutritional value and making sure people get the nutrients they need for optimum health are the goals. An essential step in improving baked food's nutritional value and raising customer satisfaction is determining the extent and necessity of enrichment. Hence, the present study was carried out on the enrichment of proso millet cookies with different proportions of oyster mushroom powder. The dried oyster mushroom powder was incorporated into the cookies with varying proportions ranging from 6-14 per cent. The sensory evaluation revealed that the cookies with 12 per cent were found most acceptable for all sensory parameters as compared to other treatments. The cookies with 12 per cent oyster mushroom flour had 11.25 per cent protein, 5.09 per cent crude fiber, 5.11 per cent insoluble dietary fiber, 1.96 per cent soluble dietary fiber and 452 kcal energy. The present study concluded that the oyster mushroom powder in millet cookies can be incorporated up to 12 per cent imparting good sensory and nutritional properties of cookies.

1. Introduction

Mushrooms are often referred as 'white vegetables' or 'boneless vegetarian meat'. They possess significant nutritional and medicinal properties and are sometimes referred as nutraceuticals (Schachter *et al.*, 2005). The oyster or pearl oyster mushroom, also known as *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. has the second position in the cultivation of edible mushrooms worldwide after *Agaricus bisporus* (Sanchez, 2009). Oyster mushrooms are packed with protein (20-25%), fiber (13-24%) on dry weight basis, lipids (4-5%), carbohydrates (37-48% on a dry basis) and ash content (8-13%) (Alam *et al.*, 2008). *Pleurotus* species are rich sources of proteins, minerals such as calcium, iron, phosphorus, sodium and potassium, and important B-complex vitamins such as thiamine, riboflavin, folic acid, and niacin (Caglarirmak, 2007). Oyster mushrooms are low in calories, fat-free, cholesterol-free, gluten-free and very low in sodium. Eating oyster mushrooms regularly appears to decrease the risk of obesity, diabetes, cancer and heart disease and increase the immunity system of the body (Maurya *et al.,* 2018). Adding oyster mushrooms in dried powder form to ready-to-eat bakery products like cake, bread, biscuits and cookies can enhance their protein and fiber content as well as the mineral composition of products, making them more nutritious.

Proso millet, *Panicum miliaceum* (Jacq. ex Fr.) P. Kumm. is a minor millet which is having the highest protein content among minor millets. Minor millets are underutilized in the baking industry because of their gluten-free nature. But the millets can be a potential ingredient for baked products making them more and more nutritious. The golden yellow colour, high starch and high protein content of proso millet make it a suitable choice as baking flour. The present study was conducted in 2022 at Department of Food and Nutrition, University of Agricultural Sciences, Dharwad, Karnataka. The white variety of oyster mushrooms (*P. ostreatus*) and proso millet (*P. miliaceum*) were used in the present study to develop cookies.

2. Materials and Methods

2.1 Protocol to make oyster mushroom powder

The protocol for drying oyster mushrooms and preparing powder was carried out according to the process given by Verma *et al.* (2023). The prepared oyster mushroom powder was packed in zip-lock covers and stored at refrigeration condition for further incorporation in cookies.

2.2 Enrichment of proso millet cookies with oyster mushroom powder

The oyster mushroom powder was incorporated in the proso millet cookies formulations with varying proportions as given in Table 1.

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The best-accepted proso millet cookie formulation with 80 per cent proso millet was selected from the previous sensory trials for further enrichment with oyster mushroom powder. The 80 per cent proso millet formula was taken as the control formula which was enriched with varying ratios of oyster mushroom powder from 6 to 14 per cent on a dry weight basis, keeping other ingredients (baking soda, baking powder and vanilla essence) constant for all treatments. The cookies were baked at 180°C for 15 min and let to cool down to room temperature after which cookies were packed in double-layered aluminium pouches.

Table 1: Enrichment of proso millet cookies with oyster mushroom powder

Treatment	Proso millet flour (g)	Refined wheat flour (g)	Oyster mushroom powder (g)	Fat content (g)	Sugar content (g)
Control	80	20	0	60	50
T1	80	14	6	60	50
T2	80	12	8	60	50
T3	80	10	10	60	50
T4	80	8	12	60	50
T5	80	6	14	60	50

2.3 Physical characteristics of oyster mushroom enriched proso millet cookies

It is crucial to assess the physical attributes of ingredients as they directly impact the quality aspects of the final product. Therefore, it is imperative to analyze the physical properties of ingredients before incorporating them into products.

2.3.1 Weight of cookies

To measure the weight of the oyster mushroom cookies, an electronic weighing balance was used, which had a high sensitivity of 0.01 g. This ensured that even the slightest variation in weight was accurately measured.

2.3.2 Volume of cookies

To determine the volume of the oyster mushroom cookies, a 25 ml measuring cylinder was used. 10 g of sample were poured into the cylinder, which was then tapped on a surface ten times from a height of 8-10 cm. The resulting volume was noted in millilitres.

2.3.3 Bulk density of cookies

Finally, the bulk density of the cookies was analyzed using the Okaka and Potter (1979) method. This involved dividing the weight of the sample by its volume, using the following formula:

Bulk density = Flour weight (g)/Flour volume (ml).

By using this method, the bulk density of the cookies was determined in g/ml providing valuable insights into the physical properties of the ingredients used in the production process.

2.3.4 Diameter of cookies

The diameter of cookies was calculated according to the process of AACC (2000). The diameter of six cookies was recorded by keeping them edge to edge and again after rotating each cookie to 90° angle, then the average diameter was calculated by dividing the diameter of six cookies by the number of cookies. The diameter was denoted in cm.

2.3.5 Thickness of cookies

The thickness of cookies was calculated according to the process of AACC (2000). The thicknesses of six cookies stacked on top of each other in different orders were measured and the average thickness was obtained by dividing the total thickness by the number of cookies. The thickness was denoted in cm.

2.3.6 Spread ratio and spread factor

The spread ratio of cookies represents the ratio of diameter to height. The diameter and thickness of six randomly selected cookies were calculated with the help of a measuring scale and callipers. The reading was repeated in triplicates by rotating the cookies to a 90° angle. The two parameters were calculated by the following formulas:

Spread ratio = Cookie diameter/Cookie thickness

Spread factor = Cookie diameter/Cookies thickness × 100

2.3.7 Colour of cookies

The evaluation of color was conducted using a spectrophotometer model (Konica Minolta CM 2600/2500). The analysis was based on the L* value, which denotes the degree of lightness or darkness on a scale of 0 (black) to 100 (white), the* value, which represents the degree of redness (+a) to greenness (-a), and the b* value, which depicts the degree of yellowness (+b) to blueness (-b). These three chromatic components were utilized to assess the colour attributes (CIE, 2004).

2.3.8 Textural properties of cookies

The textural properties of cookies, such as hardness and fracturability, were analyzed using a texture profile analyzer after production. To perform the analysis, the penetration test was carried out using a 2 mm P/2 cylindrical probe and exponent software. The penetration test measures the depth of penetration, or the time required to reach a certain depth, under a constant load. Hardness and fracturability were used to represent the firmness of cookies during the penetration test. The area under the curve was taken as an indicator to compare the hardness and fracturability of cookies. The hardness of cookies was represented by force (g), while linear distance was used as an indicator of fracturability (mm) (Patil *et al.,* 2011).

2.4 Nutritional composition of oyster mushroom enriched proso millet cookies

The nutritional composition includes macronutrients, micronutrients, phytochemicals and antioxidants present in the cookies that are beneficial to human health.

2.4.1 Moisture

To determine the amount of moisture present in the sample, 10 g of the cookies sample was put into a moisture cup and heated at 105°C until the weight of the moisture cup remained stable. After each heating cycle, the moisture cup was cooled down in a desiccator before taking measurements. The moisture content of the sample was reported as g/100 g of the sample according to AOAC (2019). The formula for calculating the moisture content is as follows:

Moisture content (%) = [{original weight (g)-final weight (g)}/weight of the sample (g)] × 100.

2.4.2 Protein

The protein content of a cookie sample can be calculated as a percentage of total nitrogen using the Micro Kjeldhal method (AOAC, 2019). To carry out this method, the sample was digested using the Kelplus-Classic Dx (Pelican equipment) digestion unit, which automatically carries out the distillation process. Using the following formula, the nitrogen content is then determined:

Nitrogen content of sample (%) = [(1.4 × Normality of the acid × titrant value) / Sample weight (g)] × 100

To calculate the protein content as a percentage, we need to multiply the nitrogen content (%) by the nitrogen-to-protein conversion factor, which is 6.25. The formula to calculate the protein content is:

Protein content (%) = 6.25 × Nitrogen content (%)

2.4.3 Fat

The determination of the fat content in the sample was done using the solvent extraction method. In this method, a thimble was filled with a precisely weighed 5 g dry sample. The thimble was then placed in a Socs Plus apparatus, and anhydrous ether was used to extract the sample for approximately an hour. After the evaporation of ether, the residue in the flask was dried in an oven at temperatures ranging from 80-100°C. Once dried, it was cooled in a desiccator and weighed, as per AOAC (2019). The fat content (%) was calculated using the formula:

Fat (%) = [{Final weight of beaker(g) – Initial weight of beaker (g)} /Weight of the sample (g)] × 100

2.4.4 Crude fiber

The crude fiber content of a sample was estimated using the gravimetric method. To do this, the moisture and fat-free sample underwent hydrolysis with acid followed by alkali. The residue that remained after the final filtration was dried in a hot air oven and weighed followed by incineration in a muffle furnace and reweighing. To determine the crude fiber content of the sample, the following formula given by AOAC, 2019 was used:

Crude fiber (%) = [(crucible weight after drying – crucible weight after ashing) / weight of the sample (g)] × 100.

2.4.5 Ash

To determine the total amount of ash, 5 g of the sample was added to a crucible. The sample was heated for 4-5 h in a muffle furnace at 600°C. After heating, it was allowed to cool down and weighed. This process was repeated twice using the same weights, resulting in nearly white or greyish-white ash (AOAC, 2019). The ash content was calculated using the formula:

Ash content (%) = [(crucible weight with sample before ashing - crucible weight with ash after ashing)/sample weight (g)] × 100

2.4.6 Carbohydrate estimation

The carbohydrate content was determined using the difference method given by AOAC (2019). To calculate g/100 g of total carbohydrates, values of all proximates except crude fibre were subtracted from 100. Similarly, to calculate g/100 g of available carbohydrates, the sum of all proximates including crude fibre was subtracted from 100.

2.4.7 Total calorific energy

According to the AOAC (2019), the calorific value of a sample was calculated by multiplying the percentages of carbohydrates, protein, and fat with their respective water constants, which are 4, 4, and 9. The total energy of the sample was then obtained by adding the products of each percentage with their respective water constant. This value is expressed per 100 g of the sample.

2.4.8 Dietary fiber estimation

The analysis of the dietary fiber fractions, including soluble, insoluble, and total, was conducted using an enzymatic-gravimetric method. The estimation process involved the use of an amyloglucosidase enzyme as per the AOAC (2019).

2.5 Mineral estimation

2.5.1 Macromineral

The calcium content was determined by precipitating calcium as calcium oxalate and titrating the oxalate solution in diluted acid against standard potassium permanganate, as described in AOAC (2019). The formula used to calculate the amount of calcium was as follows:

Calcium (mg/100 g) = [(Volume × 0.2004 × titre value)/(sample weight x aliquot)] × 100

2.5.2 Microminerals (mg/100 g)

To estimate the trace elements such as iron, zinc, copper, and manganese present in the sample, wet digestion with a triacid mixture was used. Subsequently, the microminerals such as Cu, Mn, Zn, and Fe in a known aliquot of the test sample that had been diluted were measured using an atomic absorption spectrophotometer, according to the AOAC (2019). Calibration was carried out using commercial standards.

2.6 Estimation of antioxidant components

2.6.1 Tannins

The tannins were analyzed through calorimetry, utilizing the Folin-Denis reagent (FDR) to observe the blue color produced by the reduction of phosphotungstomolybdic acid in an alkaline solution. The defatted sample was extracted by using 85 per cent methanol and 1% hydrochloric acid for 30 min with periodic shaking. After filtering the extract, the absorbance of the filtrate was measured at 760 nm. The results were denoted in tannic acid equivalent (TAE) per 100 g of sample, using tannic acid (Schander, 1970) as the standard.

2.6.2 Total phenolic content (TPC)

The estimation of total phenolic content was done using the Folin-Ciocalteau reagent. The sample was extracted using 80 per cent

ethanol, and the supernatant was utilized for estimation after evaporation. The measurement of absorbance of the sample was taken at 650 nanometres and results were denoted as mg of gallic acid equivalent per 100 g of sample, as per Ranganna's method in 1986.

2.6.3 Antioxidant capacity

The antioxidant capacity was analyzed using a method based on electron transfer, known as the DPPH (2, 2-diphenyl-1-picryl-hydrazyl), a standard for free radical scavenging activity. This method produces a violet-coloured solution in methanol. To carry out the antioxidant activity analysis, the spectrophotometric approach with DPPH was utilized, as per the AOAC guidelines of 2019.

2.6.4 Sensory evaluation of cookies

Fifteen semi-trained individuals evaluated the sensory qualities of the cookies using a 9-point hedonic scale. The panellists assessed attributes such as appearance, colour, taste, texture, flavour and overall acceptability. The cookies' acceptability was determined using the following formula:

Acceptability index = (sum of scores for all attributes/Total of the max score for each attribute) × 100

2.6.5 Statistical data analysis

The data was analyzed using MS Excel and SPSS software version 22. The results are presented in the form of mean and standard deviation. Various statistical tests like one-way ANOVA, post-hoc test, and Duncan test were performed to validate the findings.

3. Results

3.1 Sensory analysis of oyster mushroom enriched proso millet cookies

The results of the organoleptic evaluation of cookies are presented in Table 2. The overall acceptability of control cookies was 8.00. The cookies scored 8.40 for appearance, 8.33 for colour, 7.60 for taste, 8.07 for flavour and 8.07 for texture. The overall acceptability of oyster mushroom-enriched cookies ranged from 6.87 to 7.93, the highest being 10 and 12 per cent, followed by 8 (7.80) and 6 per cent (7.73) and the lowest being 14 (6.87) per cent. The mean scores were found significantly on par with each other from 6 to 12 per cent, but the difference was significant (p<0.05) at the 14 per cent level. For appearance of cookies, the score ranged from 7.07 to 8.20 the highest being in 6, 8 and 10 per cent, whereas the lowest being in 14 per cent of the oyster mushroom level. The mean scores were not significant up to 12 per cent incorporation, but decreased significantly (p<0.05) for the 14 per cent level. The scores for colour, ranged from 6.93 to 8.20, the highest being in 6 and 8 per cent with an 8.20 score, followed by 12 per cent (8.07) oyster mushroom incorporation and the lowest being in 14 per cent. The difference in mean scores was not significant up to 12 per cent, but decreased significantly by 14 per cent level. The taste scores were in the range of 6.67 to 7.87, the highest being in 8 per cent, followed by 12 per cent (7.73) and the lowest being in 14 per cent. The mean scores were not significant at a 5 per cent level up to 12 per cent, level of incorporation whereas, at a 14 per cent level it decreased significantly at a 5 per cent level. The mean scores of flavour ranged from 6.80 to 7.60, the highest (7.60) being in 8, 10 and 12 per cent followed by 6 per cent (7.53) and the lowest being for 14 per cent. The mean scores were not significantly affected by up to 12 per cent incorporation of oyster mushroom powder in cookies. The texture ranged from 7.20 to 7.80, the highest being in 12 per cent (7.80), followed by 10 per cent (7.73) and the lowest being in 14 per cent. A significant difference in the means scores was not found up to 12 per cent and the texture was on par for all treatments except the 14 per cent level of incorporation. The overall acceptability ranged from 6.87 to 7.93, the highest being in 10 and 12 per cent and the lowest in 14 per cent incorporation. The cookies with 14 per cent of mushroom scored significantly lower for overall acceptability (6.87) while up to 12 per cent (7.93) the cookies were on par with each other and also on par with control (8.00). For appearance, colour, taste, flavour and texture the 12 per cent oyster mushroom enriched cookies scored 8.13, 8.07, 7.73, 7.60 and 7.80, respectively, whereas control cookies scored 8.40, 8.33, 7.60, 8.07 and 8.07 for appearance, colour, taste, flavour, texture, respectively, which were not significantly different at 5 per cent level of significance. As the level of incorporation was acceptable up to 12 per cent it was selected to provide more nutritional value to the cookies.

Table 2: Sensory scores for optimization of oyster mushroom powder in proso millet cookies

Treatment	(PMF: RWF: OMP)	Appearance	Colour	Taste	Flavour	Texture	Overall acceptability
Control	80:20:0	8.40 ± 0.63^{a}	8.33 ± 0.62^{a}	7.60 ± 1.72^{a}	8.07 ± 0.70^{a}	8.07 ± 0.70^{a}	8.00 ± 0.65^{a}
T 1	80:14:6	8.20 ± 0.68^{a}	8.20 ± 0.68^{a}	7.67 ± 0.82^{ab}	7.53 ± 0.83^{ab}	7.73 ± 0.80^{ab}	7.73 ± 0.80^{a}
T 2	80:12:8	8.20 ± 0.56^{a}	8.20 ± 0.56^{a}	7.87 ± 0.64^{ab}	7.60 ± 0.74^{ab}	7.47 ± 0.52^{ab}	7.80 ± 0.68^{a}
T 3	80:10:10	8.20 ± 0.68^{a}	8.00 ± 0.65^{a}	7.60 ± 0.63^{ab}	7.60 ± 0.83^{ab}	7.73 ± 0.46^{ab}	7.93 ± 0.59^{a}
T 4	80:08:12	8.13 ± 0.35^{a}	8.07 ± 0.59^{a}	7.73 ± 0.59^{ab}	7.60 ± 0.63^{ab}	7.80 ± 0.77^{a}	7.93 ± 0.70^{a}
T 5	80:06:14	7.07 ± 0.88^{b}	6.93 ± 0.79^{b}	6.67 ± 0.62^{b}	6.80 ± 0.86^{c}	7.20 ± 1.01^{b}	6.87 ± 0.83^{b}
	F-values	8.26	9.25	3.23	4.22	2.47	5.30
	SEM	0.48	0.51	0.43	0.41	0.30	0.43
	CD	1.36**	1.45**	1.21**	1.15**	0.84**	1.20**

Note: Values are the mean ± SD of scores of 15 panel members. PMF: Proso millet flour, RWF: Refined wheat flour, OMP: Oyster mushroom powder. Sensory evaluation was done by a nine-point hedonic scale, SEM-Standard error mean, CD-Critical Difference, NS-non-significant, *Significant at 5 per cent, **Significant at 1 per cent. Values with a different superscript in the same column are significantly different (p<0.05).

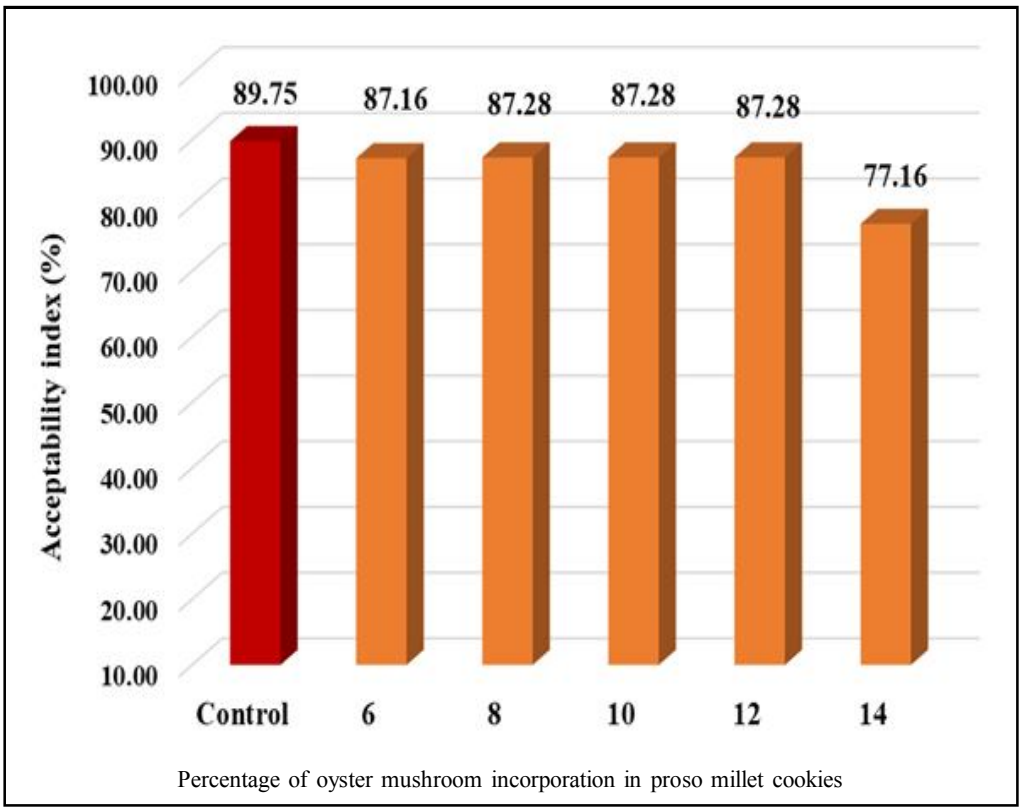

Figure 1: Acceptability indices of oyster mushroom enriched cookies in comparison with proso millet cookies.

Figure 1 depicts the acceptability indices of proso millet cookies enriched with different proportions of oyster mushrooms. The acceptability index of proso millet cookies (control) was 89.75. The acceptability index of mushroom-enriched cookies ranged from 77.16 to 87.28, the highest being in 8-12 per cent and the lowest being in 14 per cent of oyster mushroom-enriched cookies. At the 14 per cent level of incorporation, the mean sensory scores decreased at the 5 per cent level of significance. The mean sensory scores for 8, 10 and 12 per cent incorporation were found on par with each other. Therefore, to incorporate more oyster mushroom powder in the cookies, the 12 per cent formulation was selected as the best formula as it will enhance the nutritional composition of the cookies. The final accepted proso millet cookies and oyster mushroom enriched cookies are presented in Figure 2.

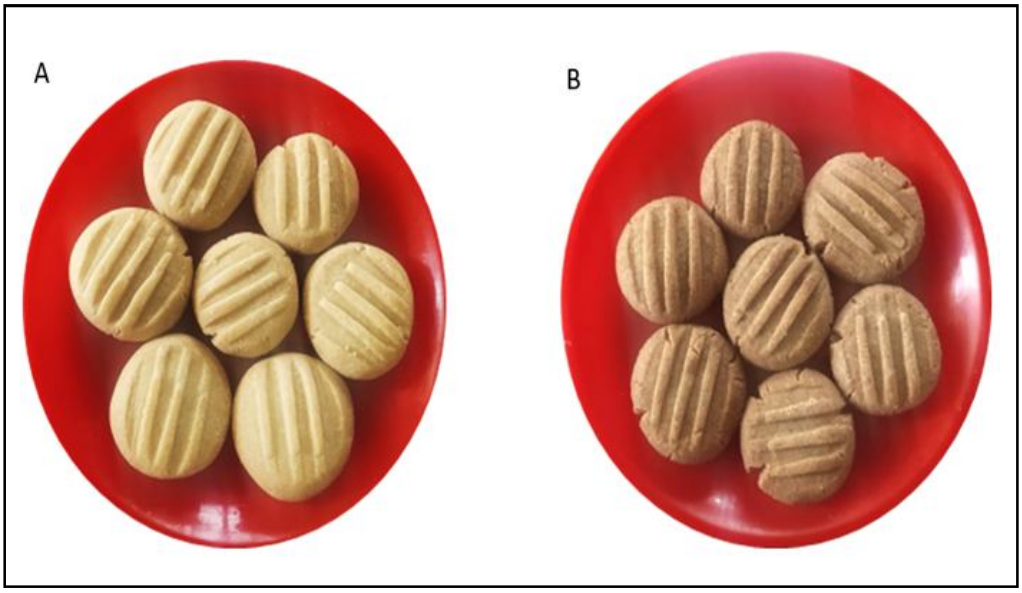

Figure 2: A. Proso millet cookies B, Oyster mushroom enriched proso millet cookies.

3.3 Physiconutritional analysis of oyster mushroom enriched proso millet cookies

3.3.1 Physical characteristics of oyster mushroom enriched proso millet cookies

Table 3 presents the physical characteristics of oyster mushroom-enriched cookies. The weight of 12 per cent oyster mushroom enriched proso millet cookies was 13.67 g. The volume of oyster mushroom cookies was 13.05 ml. The bulk density of oyster mushroom-enriched proso millet cookies was 0.77 g/ml. The diameter of the cookies was 4.13 cm. The thickness was 1.53 cm in oyster mushroom-enriched proso millet cookies. The spread ratio of oyster mushroom enriched proso millet cookies was 2.98, whereas the spread factor in oyster mushroom proso millet cookies was 270.79 per cent. The colour analysis found that oyster mushroom cookies were darker (L* 62.42), less red (a* 7.41) and less yellow (b* 21.67). The C* value defined that the intensity of the colour of the oyster mushroom was 23.11. The texture of the cookies was denoted in terms of hardness (g) and fracturability (mm). The cookies had a hardness of 2065.17 g and fracturability was 19.98 mm.

Table 3: Physical properties of oyster mushroom enriched proso millet cookies

Physical properties		Oyster mushroom enriched proso millet cookies
Weight (g)		13.67 ± 0.22
Volume (ml)		13.05 ± 0.05
Bulk density (g/ml)		0.77 ± 0.00
Diameter (cm)		4.13 ± 0.06
Thickness (cm)		1.53 ± 0.02
Spread ratio		2.71 ± 0.06
Spread factor (%)		270.79 ± 5.93
Colour values	L* (lightness)	62.42 ± 0.02
	a* (Redness)	7.41 ± 0.01
	b* (yellowness)	21.67 ± 0.15
	C* (Chroma)	23.11 ± 0.01
	H° (Hue)	71.24 ± 0.01
Textural properties	Hardness (g)	2065.17 ± 123.89
	Fracturability (mm)	19.98 ± 0.00

Note: Values are the mean ± SD of three replications.

3.3.2 Nutritional composition of oyster mushroom enriched proso millet cookies

The nutritional composition of oyster mushroom-enriched proso millet cookies was analyzed according to AOAC (2019) methods and presented in Table 4. The oyster mushroom-enriched cookies had a lower moisture content of about 12.59 per cent. The protein, crude fat, crude fiber and ash were 11.25, 21.45, 5.09 and 1.22 per cent in oyster mushroom-enriched cookies respectively. Total carbohydrate content found in oyster mushroom enriched cookies was 53.49 per cent, whereas available carbohydrate was 48.41 per cent. The cookies made with 12 per cent oyster mushroom powder were found with an energy content of 452 kcal/100 g.

3.3.3 Dietary fiber of oyster mushroom enriched proso millet cookies

The dietary fiber of oyster mushroom-enriched cookies was estimated into three categories including soluble, insoluble and total dietary fiber. Table 5 shows the dietary fiber components of oyster mushroom cookies. The total dietary fiber content of oyster mushroom cookies was 7.07 per cent which constitutes 1.96 per cent soluble and 5.11 per cent insoluble dietary fiber.

3.3.3 Mineral composition of oyster mushroom enriched proso millet cookies

Millet and oyster mushroom powder are rich in minerals that help to enhance the mineral profile of bakery products. The mineral composition of oyster mushroom enriched cookies was estimated and given in the following Table 6 for calcium, iron, zinc, copper and manganese. The oyster mushroom-enriched cookies contain 26.07 mg calcium, 2.02 mg iron, 1.05 mg zinc, 0.41 mg copper and 0.95 mg manganese.

Table 4: Proximate composition of oyster mushroom enriched proso millet cookies

Proximate (g/100 g)	Oyster mushroom enriched proso millet cookies
Moisture	12.59 ± 0.32
Protein	11.25 ± 0.25
Fat	21.45 ± 0.18
Crude fiber	5.09 ± 0.02
Ash	1.22 ± 0.00
Total carbohydrate	53.49 ± 0.72
Available carbohydrate	48.41 ± 0.74
Energy (kcal)	452 ± 0.67

Note: Values are the mean ± SD of three replications.

Table 5: Dietary fiber components of oyster mushroom enriched proso millet cookies

Dietary fiber (g/100 g) proso millet cookies	Oyster mushroom enriched
Soluble dietary fiber (SDF)	1.96 ± 0.06
Insoluble dietary fiber (IDF)	5.11 ± 0.08
Total dietary fiber (TDF)	7.07 ± 0.14

Note: Values are the mean ± SD of three replications.

Table 6: Mineral composition of oyster mushroom enriched proso millet cookies

Mineral (mg/100 g)	Oyster mushroom enriched proso millet cookies
Calcium (Ca)	26.07 ± 0.24
Iron (Fe)	2.02 ± 0.03
Zinc (Zn)	1.05 ± 0.03
Copper (Cu)	0.41 ± 0.03
Manganese (Mn)	0.95 ± 0.03

Note: Values are the mean of three replications.

3.3.4 Antioxidant properties of oyster mushroom enriched proso millet cookies

Generally, bakery products are not considered as good source of antioxidants. But this can be reversed by the addition of millets in bakery products which are good sources of tannins and phenols that contribute to the total antioxidant capacity of the product. Table 7 shows the antioxidant properties of oyster mushroom-enriched cookies. The tannin content in oyster mushroom-enriched cookies was 35.40 mg TAE/100 g. The total phenolic content in oyster mushroom cookies was 25.67 mg GAE/100 g. The antioxidant capacity (DPPH inhibition activity) of oyster mushrooms was 24.63 per cent.

Table 7: Antioxidant properties of oyster mushroom enriched proso millet cookies

Antioxidants	Oyster mushroom enriched proso millet cookies
Tannins (mg TAE/100 g)	35.40 ± 0.66
Total phenolic content (mg GAE/100 g)	25.67 ± 0.76
Antioxidant capacity (DPPH inhibition activity %)	24.63 ± 3.38

Note: The values represent the average of three replications ± standard deviation. TAE-Tannic acid equivalent, GAE-Gallic acid equivalent, DPPH-2, 2-Diphenyl-picryl-hydrazyl.

4. Discussion

The oyster mushrooms (*P. ostreatus*) are nutritious food that can provide a good amount of proteins, minerals and vitamins in the diet. To make the consumption of oyster mushrooms more convenient, it is important to value addition. Value addition involves the enrichment of food products with additional nutrients through any food substance or any food nutritive component to increase its value in terms of nutrition, palatability and convenience. The bakery products are ready to eat and more convenient to accept by consumers. To enhance the nutritive value of bakery products, many novel food substances are available that can be incorporated into products. Bakery products made with millet have high value among consumers because of their nutritional value. Incorporation of oyster mushrooms in bakery products can enhance the protein and fibre content which is lacking in the processed foods of present. Sustainable mushroom production and derivatives to produce protein foods can spark a non-green revolution (Chang, 1999). Consumers are suggested to use mushrooms with foods as a source of vegan protein for all age groups to improve diet quality. Proso millet and oyster mushrooms both are sources of good quality and quantity of protein which helps to develop a high-protein cookie. The cookies will serve a good amount of calories, protein, minerals on per serving basis (Feeney *et al.*, 2014).

Researchers found that the 12 per cent oyster mushroom incorporation by substituting the wheat flour in regular cookies was acceptable (Andhale *et al.,* 2021). The fortification of wheat flour cookies with 15 per cent of mushroom powder was acceptable and it will improve baking period quality, cookie shape, protein and dietary fiber content (Hasan *et al.,* 2020). The biscuits with 10 per cent of mushroom powder were most acceptable. Wan *et al.* (2012) reported only 2-6 per cent of incorporation was acceptable in butter cookies (Kumar and Barmaray, 2007; Wakchaure *et al.,* 2010; Okafor *et al.,* 2012; Bello *et al.,* 2018; Prodhan *et al.,* 2020) For cakes, 15 per cent addition of oyster mushroom flour was found to be optimum without affecting its sensory qualities (Sheik *et al.,* 2010). Fortification of biscuits with white button mushrooms (*A. bisporus*) was also carried out by Kumar and Barmanray (2007) who reported that the biscuits with 5 and 10 per cent mushroom powder were acceptable with a sensory score of 8.2 and 8.1, respectively. The nutrient composition showed that the protein content increased by 1 per cent with every 5 per cent of incorporation. The addition of mushroom powder imparts good color, crunchy texture and good taste in different value-added biscuits of oyster mushrooms (Wakchaure *et al*., 2010). Cookies with a higher spread ratio are most desirable as they show the ratio of diameter and height of the cookie.

In the present study, the cookies had a spread ratio of 2.71 as the thickness was 1.53 cm which shows that there was less increase in the height of cookies after baking as the proso millet flour and oyster mushroom powder were gluten-free, there was very little gluten in cookies which was contributed by a small portion of refined wheat flour used in cookie dough. The diameter of the cookie is affected by the sugar dissolution, whereas the height of the cookies shows the inhibition of gluten development. These two effects are combined in one parameter, i.e., spread ratio (Finney *et al.,* 1950). Replacing up to 20 per cent of refined wheat flour with mushroom flour and sweet potato flour increased the fiber, protein and ash content of cookies (Ibrahim and Hegazy, 2014). Up to 8 per cent incorporation of oyster mushroom powder in biscuits could develop low GI (GI=49) biscuits (Ng *et al.,* 2017). Cookies made with 15 per cent oyster mushroom flour reported, 9.46 per cent protein, 22.57 per cent fat, 1.87 per cent fiber, 1.80 per cent ash and 64.30 per cent carbohydrate (Ibrahim and Hegazy *et al.,* 2014). Similarly, the cookies with a 12 per cent incorporation level had 14.45 per cent protein, 17.22 per cent crude fat, 2.10 per cent crude fiber and 52.75 per cent carbohydrate (Andhale *et al.,* 2021) which was found on par with

the present study. One serving of oyster mushroom cookies (25 g) will provide 113 kcal energy, 2.8 g of good quality protein, 5.3 g of fat, 1.3 g of crude fiber, and total dietary fiber of 1.76 per cent.

It has been discovered that mushrooms contain a variety of substances that possess prebiotic properties, such as chitin, hemicellulose, β-glucan, α-glucan, mannans, xylans and galactans are just some of the many examples (Aida *et al*., 2009). Polysaccharides and protein-polysaccharide complexes, which are considered prebiotics, can also be found in mushrooms. These prebiotics are not broken down in the human stomach or small intestine but instead make their way to the colon, where they promote the growth of beneficial bacteria like *Lactobacillus acidophilus* and *Bifidobacterium longum* subsp. (Chou *et al*., 2013). The oyster mushroom enriched proso millet cookies are a good snack for growing children, adolescents and the heavy activity workers as it will provide a good amount of protein and dietary fiber. It is also suitable for people suffering from diabetes, hypertension, obesity and constipation as millet fiber and oyster mushroom fiber help in reducing the risk of diseases.

The most important minerals for human health are calcium as it helps to maintain bone health, iron which prevents anemia, zinc as it helps to boost the immune system and prevent infection, copper which facilitates the functions of iron and zinc, manganese which is necessary for nerve function, formation of blood clotting factor and sex hormone. Being so important for the human body, they must be supplied in the diet in good amounts. These minerals are most important for growing children, adolescents, old age people and pregnant women. The oyster mushroom contributed a good amount of antioxidant activity in the cookies even after the baking loss of some antioxidant compounds. In thermal processing, the increased level of polyphenols is because of the release of the bound form of polyphenols or the formation of melanoidin, a by-product of the maillard reaction which gives high antioxidant activity (Azad *et al.,* 2019). The polyphenolic compounds also contribute as antioxidants that scavenge the free radicals that form during the oxidation of other biomolecules which cause cell damage. This will help to maintain oxidative stress in the body, boost immunity, prevent chronic diseases and inflammation, maintain heart health and prevent cancer. Thus, the study found that the enrichment of cookies with oyster mushroom powder enhanced the protein, fiber and mineral content of cookies making them a healthy snack for growing children, adolescents and adults. The cookies resemble refined wheat flour cookies and can be replaced with normal refined wheat flour cookies at an affordable price. One serving of oyster mushroom cookies (25 g, 2 cookies) will provide 113 kcal energy, 2.8 g of protein, 5.3 g of fat, 1.3 g of crude fibre and dietary fiber of 1.76 per cent. The developed cookies were found with the goodness of millet and oyster mushroom together.

5. Conclusion

The addition of dried mushrooms to food can increase its nutritional value and promote mushroom consumption. By processing the proso millet and mushroom, their demand, adaptability and acceptability by consumers can be increased. Drying and making powder of oyster mushrooms makes it easy to incorporate in cookies. Proso millet and oyster mushrooms both are sources of good quantity of protein which helps to develop a high-protein cookie. The cookies will serve a good amount of calories, protein, minerals per serve of cookie. Cookies made with 80 per cent proso millet and 12 per cent oyster mushroom powder were found acceptable for their sensory parameters and can be included as a good source of protein and minerals in their daily diet. The findings of the present study reported that the baked products can also be served with a good amount of nutrient and antioxidant properties by adding the goodness of millet with mushrooms.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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