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Original Article

Nutritional and Sensory Characteristics of Steamed Brownies When Substituting Wheat Flour (*Triticum aestivum* L.) with Oyster Mushroom Flour (*Pleuiotus ostreatus*)

Tatang Sopandi[1*], Fifi Alaida Putri[1], Wardah[2]

[1]*Studi Program of Biologi, Faculty of Sains and Technology, Universitas PGRI Adi Buana Surabaya , Jl. Dukuh Menanggal XII No. 17, Surabaya. East Java, Indonesia*
[2]*Program Studi Agroindustri Fakultas Vokasi, Universitas 17 Agustus 1945 Surabaya, Jl. Semolowaru No. 45, Surabaya, East Java, Indonesia*

[1]*Corresponding Author : tatang.sopandi@unipasby.ac.id*

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Abstract *- Consumer behaviour that wants healthy food encourages producers to design products with better nutritional composition and health benefits. Wheat flour, as the main ingredient for bakery products, lacks lysine, has high gluten content associated with celiac disease, and has a high glycemic index. This current study aims to evaluate the content of protein, fat, carbohydrates, and crude fiber as well as the overall properties of sensory, color, taste, aroma, and texture of steamed brownies due to the substitution of wheat flour (Triticum aestivum L.) with oyster mushroom flour (Pleurotus ostreatus) in the dough formula. The current study was conducted using a completely randomized design with 5 treatments in the proportion of substituting wheat flour with oyster mushroom flour: 0%, 5%, 10%, 15%, and 20% in the dough formulation for making steamed brownies, each of which was repeated 5 times. The study's findings indicated that the substitution of wheat flour with 5-20% oyster mushroom flour considerably ($p<0.05$) increased the crude protein, crude fat, and crude fiber content while considerably ($p<0.05$) decreasing the carbohydrate content of steamed brownies. Substituting wheat flour with 5-20% oyster mushroom flour in the dough formula also increased the panelists' acceptance of the overall properties, color, taste, and texture, but at a proportion of 20%, it decreased their acceptance of the aroma of steamed brownies. The current study concluded that wheat flour could be substituted with 15% oyster mushroom flour in the dough formulation for making steamed brownies.*

Keywords *- Nutrition, Oyster mushrooms, Sensory, Steam brownies, Wheat.*

1. Introduction

Malnutrition is a universal public health problem for children and adults globally [1], including in Indonesia, which is reported to have a prevalence of 8.27% [2]. It also hinders global poverty eradication, productivity, and economic growth [3]. Malnutrition occurs when a diet lacks proper nutrition or has an imbalance in nutrients [4]. Developing bakery products fortified with protein is one strategy to fight malnutrition [5, 6]. Mixing protein from different sources without reducing food quality can overcome malnutrition problems [7]. For manufacturers, diversifying bakery products can increase sales volume and financial profits. Edible mushrooms are rich in proteins, carbohydrates, minerals, and other nutritional compounds and have desirable textures and tastes [8]. Brownies are a type of brown cake popular with the public because of their delicious taste and soft texture. In 2020, the consumption of brownies reached 77,168 per capita per unit [9]. Brownies have advantages over other bakery products: a delicious taste and a distinctive chocolate aroma. Besides the benefits, brownies also have disadvantages, such as a low protein content (around 6%), so high protein ingredients are needed to make brownies [10]. Wheat flour is the main ingredient in brownies, which must be imported because Indonesia is not a wheat-producing country. Substituting wheat flour with local raw materials is one of the efforts to reduce dependence on wheat, especially in bakery production.

Wheat flour (*Triticum aestivum* L.) is the primary cereal feedstock in most bakery products, which contains other macro- and micronutrients, including vitamins and minerals [11]. However, the use of wheat flour as the main ingredient for bakery products is incapacitated because it is an imported

commodity for non-wheat-producing countries. Wheat flour lacks lysine, has a high gluten content associated with celiac disease, and has a high glycemic index related to diabetes, which increases blood sugar levels quickly after consumption [6]. Consumer behaviour that wants healthy food encourages producers to design products with better nutritional composition and health benefits [12].

Producing new bakery variants with high functional value, such as cakes rich in dietary fiber, bioactive agents, and nutraceuticals, is of increasing interest to producers and consumers [4]. The obstacle in substituting wheat flour with other raw materials, such as rice flour and cassava flour, in making steamed brownies is the difference in texture and softness of the final result, which can affect the taste and consistency of the brownies.

Mushrooms contain various bioactive components that can also enhance the health and well-being of food [13]. Mushroom fortification in cookies helps improve the quality of the cookies while meeting nutritional needs [14]. Several researchers report considerable attentiveness to using mushrooms as a functional ingredient to improve the nutritional profile of foods such as bread, cakes, and meat [15].

The texture and appearance of cakes fortified with less than 15% *Pleurotus eryngii* flour are acceptable to consumers [8]. Mushroom flour in portions can be used to strengthen bread and cakes without affecting their physicochemical and sensory properties [15, 16, 17]. The crispiness, color, aroma, and taste of cookies are improved when 15% shiitake mushroom (*Lentinula edodes*) flour is substituted for wheat flour.

The white oyster mushroom (*Pleurotus ostreatus*) is a mushroom that is widely cultivated and famous in the world [18], including in Indonesia, because of its preferred taste, high nutritional value, and medicinal properties [19]. The bioactive components in white oyster mushrooms exhibit immunostimulating, anti-neoplastic, anti-diabetic, anti-atherosclerotic, anti-inflammatory, hepatoprotective, and antioxidant properties [20,21]. *P.ostreatus* mushroom is one of the essential sources of β-glucan [22], with a total glucan concentration of 25.636 g/100 g dry weight and β-glucan concentrations ranging from 24.230 g/100 g dry basis [23]. The total concentration of β-glucan in *P. ostreatus* fruit bodies reached 9 g/100 g on a dry basis and in the mycelium around 4.6 g/100 g [24].

The mushroom *P. ostreatus* has become well-known and valuable as a functional food ingredient because it is free of cholesterol and low in calories, carbohydrates, fat, and sodium [25]. Extracts from *Pleurotus* mushroom species can be used to produce food supplements [26]. However, adding mushrooms should not affect functional foods' desired appearance, texture, taste, or shelf-life [8]. Substituting wheat flour with oyster mushroom flour in the dough formulation of steamed brownies can increase their nutritional value and consumer interest, support more environmentally friendly food production by reducing land and water use, and increase the utilization of agricultural waste.

Meanwhile, in terms of taste, oyster mushroom flour can add a unique flavour dimension with a strong umami flavour, which balances the sweetness of brownies and provides a more complex and interesting flavour profile. The impact of oyster mushroom flour on steamed brownies' nutritive and sensory qualities is still mostly unknown. The present study assessed the effect of oyster mushroom flour instead of wheat flour on steamed brownies' nutritional and sensory characteristics.

2. Materials and Methods

2.1. Making Steamed Brownies

The current study was carried out experimentally using a completely randomized design with 5 treatments of replacing Wheat Flour (WF) with Oyster Mushroom Flour (OMF) with a proportion of 0% (P_0), 5% (P_1), 10% (P_2), 15% (P_3), and 20% (P_4) in the dough of the steamed brownie formulation. The formulation for substituting WF with OMF in the current study is presented in Table 1.

Table 1. Formulation and proportion of substituting of wheat flour (WF) with white oyster mushroom flour (OMF)

Raw material	The formula of dought steam brownies				
	P_0	P_1	P_2	P_3	P_4
WF (g)	250	237.5	225	212	200
OMF (g)	0	12.5	25	37.5	50
CB (g)	350	350	350	350	350
WEF (g)	520	520	520	520	520
Butter (g)	380	380	380	380	380
GS (g)	250	250	250	250	250

The raw materials are 5,622.5 g of Wheat Flour (WF), 625 g of Oyster Mushroom Flour (OMF), 8,750 g of Chocolate Bars (CB), 2,600 g of Whole Egg Flour (WEF), 9,500 g of butter, and 6,250 g of Granulated Sugar (GS) purchased from local supermarkets in Surabaya, Indonesia. Each ingredient is weighed according to its proportion in the formulation for making brownies.

First, the eggs and granulated sugar are mixed evenly, then the melted butter and chocolate are added and stirred until well blended. Lastly, WF and OMF, according to the formula for each treatment, are mixed and stirred evenly.

Next, put the mixture into a 25 cm x 25 cm x 6 cm tin pan smeared with butter beforehand. The baking sheet containing the dough is put into the preheated boiler to be steamed at 170°C for 50 minutes.

2.2. Analysis of Nutritional Composition

Wheat Flour (WF), Oyster Mushroom Flour (OMF), and steamed brownies were dried to a moisture content of around 12% before analysis for nutritional composition, namely crude protein, crude fat, carbohydrates, and crude fiber. The determination of water content at 105ºC overnight, crude protein by the Kjeldahl method, crude fat Soxhlet method with hexane as a solvent, carbohydrates by difference method, and crude fiber [27]. Analysis of total dietary fiber, insoluble dietary fiber, and soluble dietary fiber analysis by different methods [27].

2.3. Sensory Assessment

A 40-person untrained panel made up of professors and students from the Faculty of Science and Technology at Universitas PGRI Adi Buana in Surabaya, Indonesia, evaluated the sensory qualities of the steamed brownies following the guidelines of Ocampo and Usita [28]. All panelists were cookie lovers, did not smoke, were not colorblind, had a taste threshold of 10% sugar in tea, and were not allergic to foods made from mushrooms. Panelists were requested to evaluate the color, taste, and aroma of brownie samples from each treatment that were cut into 3 x 3 cm cubes and given a three-digit code. The assessment scale uses a 5-point Likert scale, namely, 1 = not like/not accepted, 2 = moderately like/moderately accepted, 3 = like/accepted, 4 = like much/much accepted, and 5 = like very much/very much accepted. Sensory testing was conducted in a controlled room to minimize external factors such as odour or sound that could affect panelists' perceptions without disturbance and with sufficient lighting and stable temperature. Brownie samples with uniform shape and cold but not yet hard were served randomly.

2.4. Data Analysis

The student's t-test was used to compare the crude protein, carbohydrate, crude fat, and crude fiber content of white oyster mushroom flour and wheat flour at a considerable level of 0.05. A one-way analysis of variance at a considerable level of 0.05 was used to examine the observed data on the impact of substituting white oyster mushroom flour for wheat flour on the crude protein, carbohydrate, crude fat, and crude fiber content of steamed brownies. If the analysis of variance results were considerable, additional tests were conducted using the Tukey test at a considerable level of 0.05.

Data from panelists' assessments of the sensory characteristics of steamed brownies was carried out using Chi-square analysis with categories 1.00-1.79 = not like/not accepted, 1.80-2.59 = moderately like/moderately accepted, 2.60-3.39 = like/accepted, 3.40-4.19 = like much/much accepted, and 4.20-5.00 = like very much/very much accepted. All data analysis was performed using the SPSS 24 application.

3. Results and Discussion

3.1. *The Nutritional Composition of Wheat Flour and White Oyster Mushroom Flour*

The study's findings (Figure 1) display that there are differences in nutritional composition between Wheat Flour (WF) and white Oyster Mushroom Flour (OMF). At the same water content, the content of crude protein, crude fat, and crude fiber in OMF was significantly higher than that of WF. However, OMF carbohydrate content was considerably lower than WF.

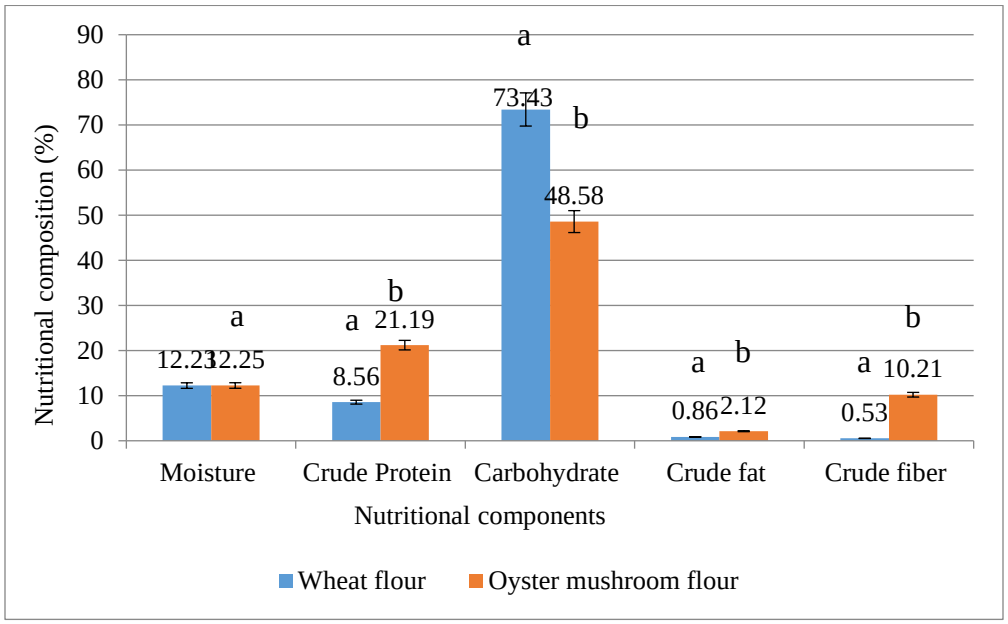

Fig. 1 The nutritional composition of the raw materials for wheat flour and oyster mushroom flour. The mean values with error bars in the same nutrient component and marked with different letters indicate considerable differences ($p<0.05$)

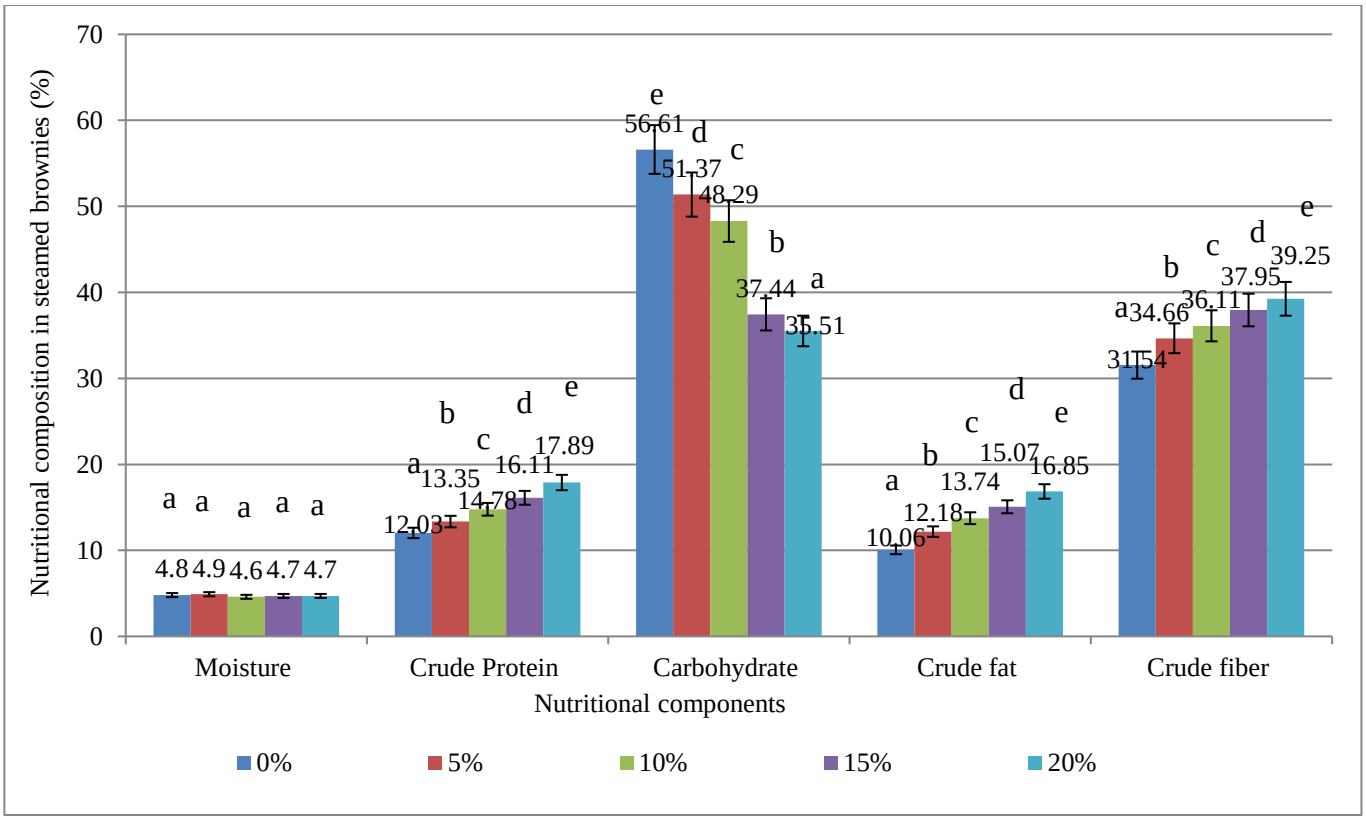

Fig. 2 The nutritional composition of steamed brownies with the proportion of oyster mushroom flour in different dough formulas. The mean values with error bars in the same nutrient component and marked with different letters indicate considerable differences ($p<0.05$)

3.2. The Nutritional Composition of Steamed Brownies

The study's findings (Figure 2) show that substituting WF with OMF in the dough formula significantly increased steamed brownies' crude protein, crude fat, and fiber content but decreased carbohydrate content considerably. The crude protein content of steamed brownies in formula 20% OMF was considerably higher than those in formulas 0%, 5%, 10%, and 15%. The crude protein content of steamed brownies in formula 15% OMF was considerably higher than those in formulas 0%, 5%, and 10%. The crude protein content of steamed brownies in formula 10% OMF was considerably higher than in formulas 0% and 5%. The crude protein content of steamed brownies in formula 5% OMF was considerably higher than in formula 0%. The carbohydrate content of steamed brownies in the 20% OMF formulation was considerably lower than in the 0%, 5%, 10%, and 15%. The carbohydrate content of steamed brownies in the 15% OMF formula was significantly lower than in the 0%, 5%, and 10%. The carbohydrate content of steamed brownies in the 10% OMF formula was considerably lower than in 0% and 5%. The carbohydrate content of steamed brownies in 5% OMF was considerably lower than in 0%. The crude fat content of steamed brownies in 20% OMF was considerably higher than 0%, 5%, 10%, and 15%. The crude fat content of steamed brownies in 15% OMF was considerably higher than 0%, 5%, and 10%. The crude fat content of steamed brownies in formula 10% was considerably higher than 0% and 5%. The crude fat content of steamed brownies in 5% OMF was considerably higher than in 0%. The crude fiber content of steamed brownies in 20% OMF was considerably higher than in 0%, 5%, 10%, and 15%. The crude fiber content of steamed brownies in 15% OMF was considerably higher than in formula 0%, 5%, and 10%. The crude fiber content of steamed brownies in 10% OMF was considerably higher than in 0% and 5%. The crude fiber content of steamed brownies in 5% OMF was considerably higher than in 0%.

3.3. Sensory Characteristics of Steamed Brownies

The study's findings (Figure 3) display that panelists can accept the overall sensory properties of steamed brownies in different categories when substituting WF with OMF. Panelists' acceptance of the overall sensory properties of steamed brownies in the OMF 0% was considerably lower compared to OMF 10%, 15%, and 20% but not considerably different compared to OMF 5%. Panelists' acceptance of the overall sensory properties of steamed brownies in OMF 10% was not considerably different compared to 15%, but both were considerably lower than 20%. The overall sensory properties of steamed brownies in 0% and 5% OMF were included in the like/accepted category, 10-15% were like much/much-accepted category, and 20% were like very much/very much accepted category.

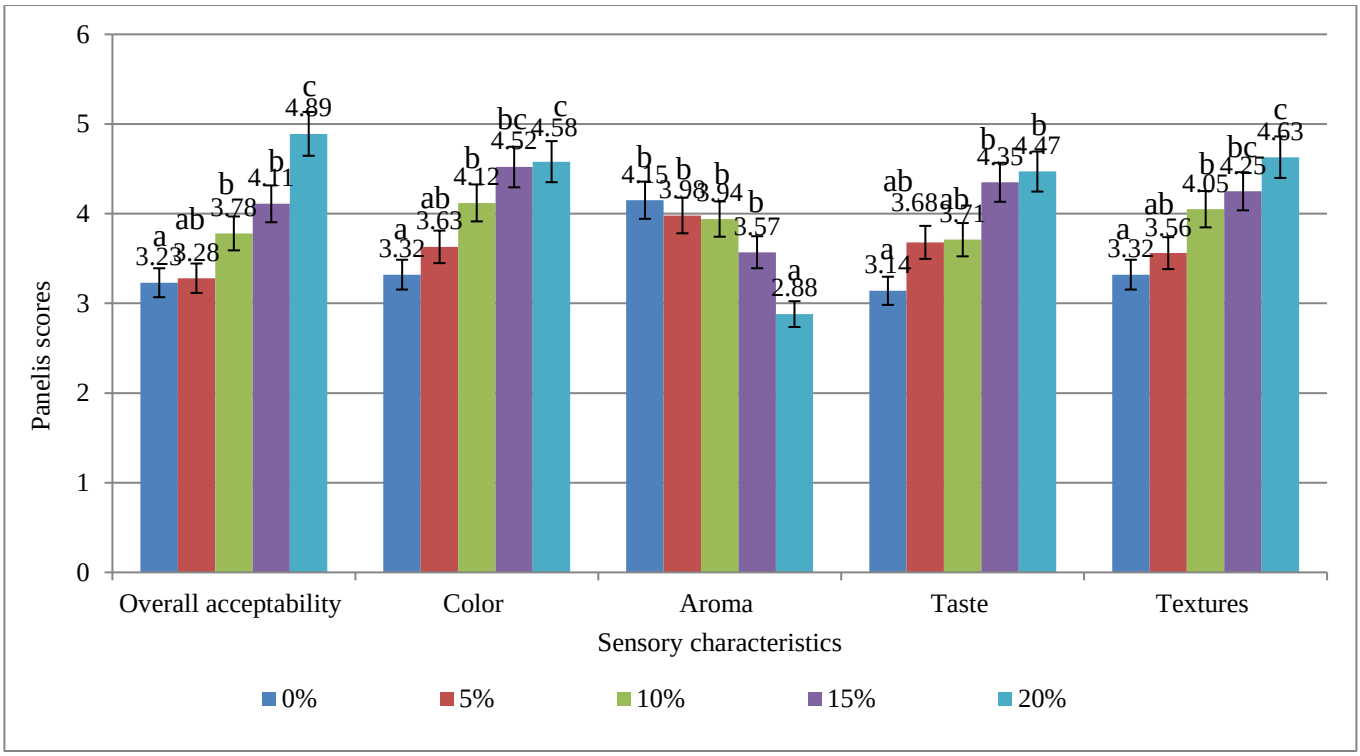

Fig. 3 Sensory characteristics of steamed brownies with the proportion of oyster mushroom flour in different dough formulas. The mean values with error bars in the same nutrient component and marked with different letters indicate considerable differences ($p<0.05$)

Panelists' acceptance of the color of steamed brownies at 0% OMF was significantly lower compared to 10%, 15%, and 20% OMF but not considerably different compared to 5% OMF. Panelists' acceptance of the color of steamed brownies at 5% OMF was not considerably different compared to 10% and 15% but considerably lower compared to 20%. The color of steamed brownies at 0-5% was included in the like/accepted category, 5-10% was like much/much-accepted category, and 15-20% was like very much/very much accepted category.

The study's findings display that increasing the proportion of OMF to substitute wheat flour reduces the aroma of steamed brownies. Panelists' acceptance of the aroma of steamed brownies at 0% OMF was not considerably different compared to 5%,10%, and 15%, but considerably higher than 20%. The aroma of steamed brownies at OMF 0%, 5%, 10%, and 15% was included in the like much/much-accepted category, while at 20%, it was included in the like/accepted category.

Panelists' acceptance of the taste of steamed brownies at 0% OMF was not considerably different compared to 5% and 10% but considerably lower compared to 15% and 20%. There was no considerable difference between panelists' acceptance of the taste of steamed brownies at 5%, 10%, 15%, and 20%. The taste of steamed brownies at 0% OMF was included in the like/accepted category, 5-10% were in the like much/much-accepted category, and 15-20% were in the like very much/very much accepted category.

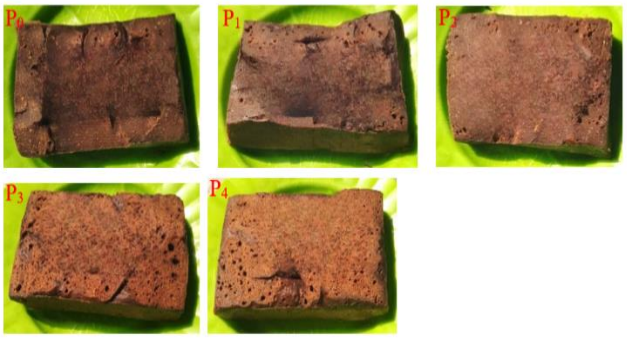

Fig. 4 The appearance of steamed brownies in different proportions of white oyster mushroom flour in formulas P_0 (0%), P_1 (5%), P_2 (10%), P_3 (15%), and P_4 (20%)

Panelists' acceptance of the texture of steamed brownies at 0% OMF was considerably lower than 10%, 15%, and 20% but not considerably different compared to 5%.

Panelists' acceptance of the texture of steamed brownies at 10% OMF was considerably lower than 20% but not considerably different compared to 5% and 15%. Panelists' acceptance of the texture of steamed brownies at 15% OMF was not considerably different compared to 20%.

The texture of steamed brownies at 0% OMF was included in the like/accepted category, 5-10% OMF were in the like much/much-accepted category, and 15-20% were in the like very much/very much accepted category.

4. Discussions

The study's findings demonstrated that the crude protein, fat, and fiber content of OMF were higher than WF's. However, the carbohydrate content of OMF is lower than WF's. The nutritional composition of OMF in this study was close to or almost the same as that of OMF, which several researchers have extensively documented. For example, Tolera and Abera [29] reported that OMF contained 43.64% carbohydrates, 24.99% crude protein, 2.12% crude fat, and 10.21% crude fiber. Ahmed *et al*. [30] reported that OMF contained 38.00% carbohydrates, 25.50% crude protein, 4.00% crude fat, and 25.20% crude fiber. The nutritional composition of the OMF varies depending on various factors such as strain variations, growing substrate composition, cultivation methods, harvesting phase, certain fruit body parts used for analysis, harvest intervals, and evaluation techniques [31, 32].

Several researchers have extensively documented the nutritional composition of WF. The nutritional composition of WF in the current study is close to or almost the same as the nutritional composition that several researchers have reported. Okpala and Egwu [33] reported that WF contained 78.10% carbohydrates, 14.7% protein, 2.10% fat, and 2.10% minerals. Kulkarni *et al*. [34] reported that WF contained 12.67% water content, 10.55% protein, 0.94% fat, 74.88 total carbohydrates, and 0.36% crude fiber. Ocheme *et al*. [35] reported that WF contained 14.7% protein, 72.73% carbohydrates, 1.93% fat, and 0.84% crude fiber. Nisar *et al*. [36] reported that WF contains 8.9% protein, 2.29% fat, and 1.44% crude fiber. Iqbal *et al*. [37] reported that WF contains 10.3–13.2 g of protein, 61.3–73.6% carbohydrates, 1.0–2.5% fat, and 2.7–10% crude fiber. Various factors influence variation in WF nutrient content. For example, Behrman *et al*. [38] reported that WF protein content is influenced by genetics, environment, nitrogen fertilization, and other aspects of crop management on factors such as stand density, root growth, number of tillers, and number of florets. Masood [39] reported that genetic varieties, agronomic conditions, and climate influenced WF moisture content. Randhawa *et al*. [40] found that the genetic makeup of wheat varieties, climatic conditions, and growing conditions influence WF gluten content. Rose and Pike [41] reported that lipase activity in dormant wheat affected the WF fat content during storage. Hossain *et al*. [42] reported that the crude fiber content of WF was affected by extraction rate, genotype, and environment.

The study's findings indicated that replacing 5-20% WF with OMF increased the content of protein, fat, and crude fiber while decreasing the carbohydrate content of steamed brownies. The increase in protein, crude fat, and crude fiber content of steamed brownies was caused by the higher OMF crude protein, crude fat, and crude fiber content compared to WF, as shown in Table 2. Meanwhile, the decrease in the carbohydrate content of steamed brownies was due to the OMF carbohydrate content lower than wheat flour. The study's findings align with the results of several studies that have reported increasing the protein, fat, and crude fiber content but reducing the carbohydrate content in various processed food products that partially replaced the proportion of WF with OMF in the formula. Das *et al*. [43] reported that 5-12% OMF fortification in the formula increased the protein, fat, and crude fiber content but decreased the carbohydrate content of the sponge cake. Parvin *et al*. [44] reported that replacing 5-10% WF with OMF in the formula increased the protein, fat, and crude fiber content but decreased the carbohydrate content of the noodles. Nordiana *et al*. [45] reported that replacing 5-10% WF with OMF in the formula increased the protein, fat, and crude fiber content but decreased the carbohydrate content of the pasta.

The study's findings indicate that partial replacement of WF with OMF can improve nutritional quality, especially protein and crude fiber, in bakery products, especially brownies. Some researchers argue the importance of enriching protein and dietary fiber in bakery products. Improving nutrition in bakery products is an attractive opportunity for the food industry with the trend of using alternative protein sources to increase food's protein and fiber content and improve the balance of amino acids [46]. The content of protein, dietary fiber, and bioactive compounds makes mushrooms an ideal candidate for improving the nutritional profile of foods [47]. Dietary fiber added to bakery products designed as functional foods benefits heart health and gastrointestinal pain reduces the risk of various cancers and prevents diseases [48]. Dietary fiber plays a role in ensuring good intestinal transit, preventing constipation, reducing the absorption of fat from the digestive tract, and supporting the absorption of toxins [49, 50].

The study's findings indicate that replacing 5-20% WF with OMF in the formula can increase panelist acceptance of steamed brownies' overall properties, color, taste, and texture; however, a 10–20% replacement decreases panelist acceptance of aroma. The replacement of 5% WF with OMF is acceptable to the panelists and does not differ from 0%. The increase in panelist acceptance of the overall properties, color, taste, and texture of steamed brownies as a result of replacing WF with OMF is thought to be due to the interaction or mixing of the components of WF, OMF, sugar, butter, chocolate, and eggs, causing a sensation that the panelists can receive. On the other hand, a proportion of 10–20% OMF creates an odour sensation that is unacceptable to the panelists. The current study aligns with Nordiana *et al*. [45], who reported that the sensory properties of pasta fortified with 10% OMF were acceptable to panelists. Das *et al*. [43] reported that the sensory properties of a 10% OMF-fortified sponge cake were acceptable to the panelists. Parvin *et al*. [44] reported that noodles fortified with 5% OMF did not produce an unpleasant aroma and had a better taste than noodles that were not fortified or fortified with 8% and 10% OMF. Aishah and Rosli [51] reported that replacing 2-6% OMF increased the odor of

paratha bread and cream cake. The taste and aroma of mushrooms depend on volatile and non-volatile compounds such as 50-nucleotides, free amino acids, organic acids, and soluble carbohydrates [52]. Oyster mushrooms have a unique smell that smells slightly fishy or seafood-like and are often compared to aniseed. Usami *et al*. [53] reported that methional, 1-octen-3-ol, and nonanal are the main active components of the aroma of the mushroom *P. eryngii* var. tuoliensis, and dimethyl trisulfide and 1-octen-3-ol are thought to be the main aroma active components of *P. cystidiosus* mushroom oil.

Based on nutritional and sensory characteristics, the current study indicated that replacing WF with OMF at a proportion of 15% could improve the nutritional characteristics and maintain the sensory properties of steamed brownies. The study aligns with Rosli and Aishah [54], who reported that *Pleurotus* sajor-cajun mushroom flour improves the nutritional profile and characteristic content and maintains the sensory properties of carbohydrate-based products. Salehi [55] suggests that mushroom flour has excellent potential to be used immediately as an ingredient in various food industries, such as breakfast cereals and bread, such as biscuits, cakes, and bread. According to Oyetayo and Oyedeji [56], a 20% replacement of white oyster mushroom flour for wheat flour raised the bread's protein level. It influenced the taste, texture, and aroma of the finished product.

The study's findings indicate that the substitution of wheat flour with white oyster mushroom flour can reduce the dependence of non-wheat-producing countries on the production of bread and biscuits, increase the economic value of local products and provide economic benefits to the community by creating new business opportunities and increasing the income of mushroom farmers. Reducing dependence on imported wheat by utilizing local resources such as oyster mushrooms can reduce the carbon footprint generated from import transportation. Utilizing organic waste as a growing medium in oyster mushroom cultivation can support sustainable agricultural practices. Substitution of wheat flour with mushroom flour, especially oyster mushroom flour, has the potential to lower the glycemic index (GI) of food products due to the β-glucan content in oyster mushrooms, which can slow down glucose absorption, thereby helping to control blood sugar levels and prevent blood sugar spikes.

5. Conclusion

The current study concluded that substituting wheat flour with oyster mushroom flour in the dough formula could increase steamed brownies' protein, fat, and crude fiber content but decrease the carbohydrate content. Substituting wheat flour with oyster mushroom flour improves organoleptic properties, color, taste, and texture. However, the proportion of oyster mushroom flour is more than 15%, which can reduce the aroma. The current study recommends 15% oyster mushroom flour in the dough formula for making steamed brownies. Further research is needed to determine the effect of substituting wheat flour with oyster mushroom flour on the content of amino acids, minerals, and vitamins. Exploration of mushroom flour substitution in bread and cake products can enrich texture, taste, and nutritional value, especially in gluten-free bread and cakes, focusing on increasing softness, chewiness, and fiber content. Further research on the use of mushroom flour in food products can improve taste and texture quality, offer health benefits, such as increased fiber and antioxidant content, and contribute to the cessation of food production.

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