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INNOVATION MUSHROOM NUGGETS FORTIFIED MORINGA LEAVES AS ANTIOXIDANT SOURCE

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ABSTRACT

This research aims to determine the impact of mushroom variety on the organoleptic, nutritional content, and antioxidant properties of moringa-fortified mushroom nuggets. A completely randomized design (CRD) was employed in this study, focusing on the impact of different mushroom types. The mushrooms used are oyster mushrooms (Pleurotus ostreatus), straw mushrooms (Volvariella volvacea), and enoki mushrooms (Flammulina velutipes). The result showed that all samples of mushroom nuggets were acceptable to the panellists, with levels of preference ranging from neutral to favourable. Mushroom nuggets fortified with Moringa leaves have a protein content of 14.90-15.51% and a fat content of 8.78-9.68%. The results of the data analysis showed that the control nuggets were significantly different from all the nuggets treated with different types of mushrooms in the parameters of aroma, taste, and texture. However, the treatment of the nuggets with different types of mushrooms did not show significant differences in the parameters of aroma, taste, and texture. The effects of different types of mushrooms on the protein and fat content of the nuggets were not significant ($p > 0.05$). The IC50 value of the antioxidant activity of mushroom nuggets fortified with Moringa leaves ranged from 5,5 ppm–34,5 ppm, with a very active category.

INTRODUCTION

Along with the development of science and technology, people's consumption behaviour has experienced a shift. People tend to like food that is practical, economical, and easy to obtain. One of them is nuggets. Nuggets generally made from chicken are high in protein but contain high-fat levels. Protein in chicken meat is 18%, and fat in

chicken meat reaches 25% (Direktorat Jenderal Kesehatan Masyarakat, 2018). Consuming foods high in fat will increase cholesterol levels in the blood, causing atherosclerosis and hypertension (Kirom et al., 2021).

An alternative food ingredient for making nuggets that are high in protein and low in fat is mushrooms. The mushroom types cultivated locally and consumed widely in Indonesia are oyster and straw mushrooms. Oyster mushrooms are widely consumed because they have a colour, taste, and texture that resembles chicken meat. Meanwhile, imported mushrooms are currently popular as a diet menu and are enoki (Setyawan & Kamil, 2021). Enoki mushrooms are widely used as a diet because they have the lowest fat content, precisely 0.28% (Feeney et al., 2014). Other mushrooms are also rich in nutritional content. For example, straw mushrooms have a protein content of up to 12.38% and oyster mushrooms have a protein content of 2.75% (Yuen et al., 2014).

Moringa leaves are known as a source of antioxidants. Antioxidants are substances that can neutralize the impact of free radicals. The main antioxidant compounds in Moringa leaves are phenolics and flavonoids. Most of the vitamins in Moringa leaves also have antioxidant activity (Rani et al., 2019).

The development of mushroom nugget products has been carried out (Trihaditia & Manisha, 2016), with the main ingredient being oyster mushrooms. Straw mushroom nuggets were developed by (Diniyah et al., 2015), and enoki mushroom nuggets by (Anam et al., 2020). However, functional compounds have not been added to the mushroom nuggets. Fortification of Moringa leaf flour in mushroom nuggets is carried out to increase the nutritional and antioxidant content. Therefore, a new alternative processed food is needed that is high in protein, low in fat, and has health effects, namely mushroom nuggets fortified with Moringa leaves. Food rich in antioxidants can provide solutions to improve the quality of public health (Sunarno & Djaelani, 2018).

Moreover, to determine whether the type of mushroom influences the nugget product, it is necessary to compare oyster mushroom nuggets, straw mushroom nuggets, and enoki mushroom nuggets.

MATERIALS AND METHODS

Materials

The essential ingredients used in the production of nuggets for this research are oyster mushrooms (*Pleurotus ostreatus*), straw mushrooms (*Volvariella volvacea*), and enoki mushrooms (*Flammulina velutipes*).

Production of Mushroom Nuggets Fortified with Moringa Leaves

Mushroom nugget production in this research refers to Trihaditia & Manisha (2016) and Hamidiyah et al. (2019) with modifications. Table 1 shows the formulation of mushroom nuggets fortified with Moringa leaves. In making mushroom nuggets, 250 g of each type of mushroom is blanched, squeezed and then finely chopped. The onions, salt, eggs, pepper, chicken stock and bread mixed for 5 minutes. Wheat flour, cornstarch, gelatin powder, and moringa leaf flour are added to the mixture and stirred well. Then, the nugget mixture is transferred to a baking dish lined with aluminium foil and steamed for 50 minutes. After that, the dough is removed and left at room temperature for 30 minutes. The nuggets are cut into 4.5x3x0.5 cm dimensions and stored in the refrigerator for 2 hours. Subsequently, the nugget pieces are removed from refrigeration, dipped in beaten egg, breaded in flour, redipped in beaten egg, and breaded in breadcrumbs. Last, the nuggets are fried until golden brown and served.

The process for making chicken nuggets is the same as for mushroom nuggets. However, there is no blanching process, so the chicken meat is directly ground with a grinder and some ice and water. The spices and fillers are also the same, except that Moringa leaf flour is not used to make chicken nuggets.

Table 1. Formulation of mushroom nuggets fortified with Moringa leaves

Materials	**Formulation (g)**			
	F0	**F1**	**F2**	**F3**
Chicken meat	250	-	-	-
Oyster mushroom	-	250	-	-
Straw mushroom	-	-	250	-
Enoki mushroom	-	-	-	250
Moringa leaf flour	-	5	5	5
Wheat flour	40	40	40	40
Cornstarch	75	75	75	75
White bread	87,5	87,5	87,5	87,5
Chicken stock powder	6	6	6	6
Egg	100	100	100	100
Garlic	18	18	18	18

Materials	Formulation (g)			
	F0	**F1**	**F2**	**F3**
Red onion	18	18	18	18
Pepper powder	2	2	2	2
Salt	2	2	2	2
Gelatin flour	7	7	7	7

Quality Analysis of Mushroom Nuggets Fortified with Moringa Leaves

The quality of mushroom nuggets fortified with Moringa leaves was reviewed using organoleptic assessment, protein content measurement, fat content measurement, and testing the level of strength of antioxidant activity. The organoleptic assessment was carried out with the help of 30 untrained panellists, Untirta Biology Education Department students, with aroma, taste and texture parameters on a hedonic scale ranging from 1-9 (ranging from strongly dislike, immensely dislike, dislike, slightly dislike, neutral, slightly like, like, very like, strongly like). Balai Penelitian Tanaman Rempah dan Obat (BALITTRO) measured protein and fat content. Protein content measurements were carried out using the Kjehdahl method, and fat content measurements were carried out using the Soxhlet method.

Antioxidant activity testing was done using the DPPH method at the Untirta Biological Science Laboratory. A 0.4 mM DPPH solution was prepared by dissolving 0.015 g of DPPH powder in 95 mL of methanol p.a. in a measuring flask (100 mL Pyrex), then homogenizing. Next, 0.05 g of sample was ground and put into 50 mL of methanol p.a., then filtered and became a 1000 ppm sample solution. The control solution was made by adding 1 mL of 0.4 mM DPPH in a measuring cup (10 mL Iwaki) and methanol p.a until the volume was 5 mL.

Antioxidant activity was determined by preparing a series of sample solutions at concentrations of 100, 200, 300, 400, and 500 ppm, derived from a 1000 ppm stock solution. Subsequently, each solution was supplemented with 1 mL of 0.4 mM DPPH solution and methanol p.a. to a final volume of 5 mL. The resulting mixtures were incubated in a dark environment at room temperature for 30 minutes. The absorbance of each solution was measured at a wavelength of 517 nm utilizing a UV752N UV-Vis spectrophotometer.

Experimental Design and Data Analysis

This research used a Completely Randomized Design (CRD) where all conditions, including temperature, place and materials used, were the same (homogeneous). The

independent variable is the type of mushroom. The types of mushrooms used are oyster, straw, and enoki mushrooms. The data obtained will be analyzed with the help of IBM SPSS Statistics 26. Data analysis uses ANOVA with conditions of normal and homogeneous distribution. If the treatment has a real effect, then the analysis will continue with the Tukey Test. Data that is not normally distributed and homogeneous will be analyzed using Kruskal Wallis nonparametric statistics. If the treatment has a real effect, then the analysis will continue with the Dunn Test.

The antioxidant activity of a sample is evaluated by its IC50 value, which represents the concentration needed to reduce DPPH radicals by 50%. A lower IC50 value indicates higher antioxidant activity. The IC50 is calculated from the linear relationship between the rate of DPPH radical inhibition and sample extract concentrations ranging from 100 ppm to 500 ppm. The regression equation y = ax+b obtained from the plot calculates the IC50 value, where y is 50, and the x is IC50 (Aini et al., 2021). Calculation of the IC50 value using Microsoft Excel 2021.

RESULTS AND DISCUSSION

Effect of Mushroom Type on Organoleptic Quality of Mushroom Nuggets Fortified with Moringa Leaves: Aroma, Taste and Texture

Organoleptic analysis indicated that the chicken nugget sample as a control had the highest favorability score for each organoleptic quality regarding aroma, taste, and texture compared to the mushroom nugget sample as the treatment. The results of this study corroborate the findings of Diniyah et al. (2015), indicating a greater consumer preference for chicken nuggets compared to mushroom nuggets. The results of the organoleptic assessment of mushroom nuggets fortified with Moringa leaves can be seen in **Table 2**.

Table 2. Results of the organoleptic assessment of mushroom nuggets fortified with Moringa leaves according to panelists

	F0	**F1**	**F2**	**F3**
Aroma	$7,3 \pm 1,1^{b}$	$5,4 \pm 1.9^{a}$	$5,3 \pm 1,9^{a}$	$5,6 \pm 2,0^{a}$
Rasa	$7,5 \pm 1,2^{b}$	$5,3 \pm 1,9^{a}$	$6,0 \pm 1,7^{a}$	$5,9 \pm 2,0^{a}$
Tekstur	$7,2 \pm 1,3^{b}$	$5,8 \pm 1,7^{a}$	$6,1 \pm 1,8^{ab}$	$6,0 \pm 2,1^{ab}$

Note: F0 = chicken mushroom nuggets (control), F1 = oyster mushroom nuggets, F2 = straw mushroom nuggets, F3 = enoki mushroom nuggets. Numbers in the table followed

by different superscript letters (a,b) in different columns show significant differences ($p<0.05$)

Aroma

The aroma of mushroom nuggets enhanced with Moringa leaves was favourably received by panellists, with scores ranging from 5.3 to 7.3 in organoleptic evaluations. Dunn's further test results showed that the control nuggets differed significantly from the mushroom nugget treatment. However, there were no significant differences if the mushroom nugget treatments were compared.

Aroma is a complex organoleptic attribute involving aroma compounds. A compound is aromatic if it evaporates quickly (volatile) to penetrate the sense of smell easily. It was found that panellists had a greater preference for chicken nuggets than mushroom nuggets. The reason is that chicken nuggets still have the distinctive aroma of chicken meat as the raw material (Nurlela et al., 2018). The principal aroma compounds identified in chicken breast meat are hexanal, characterized by a green-fresh odour, and 2-heptanol, which exhibits a green-cheesy-fatty odour profile (Ayseli et al., 2014). Meanwhile, one of the aroma compounds that oyster mushrooms, straw mushrooms and enoki mushrooms share is octane, which is described as having a sweet, fruity, and musty odour (Xu et al., 2019). These similarities influence the panellists' preferences for the aroma of nuggets, so they do not show a real difference even though the type of mushroom used as the raw material is different. The panellists' assessment of the three mushroom nugget treatments came to the same neutral conclusion. Conversely, the research conducted by Anam et al. (2020) indicated that enoki mushroom nuggets supplemented with seaweed flour were perceived as having a normal to unfavourable aroma profile by the sensory panellists. In this research, the blanching process was carried out on three types of mushrooms as raw materials and Moringa leaves, which was carried out before making nuggets. Blanching is heating food ingredients with a medium of water or steam at a temperature of 82-93 for 3-5 minutes. Blanching is done to remove unwanted aromas. In addition, the process of frying nuggets at high temperatures causes the aroma compounds to evaporate so that the aroma that tends to be disliked disappears.

Taste

The taste of mushroom nuggets enriched with Moringa leaves was favourably received by panellists, with scores ranging from 5.30 to 7.50 in organoleptic evaluations.

Dunn's further test results showed that the control nuggets differed significantly from the mushroom nugget treatment. However, there were no significant differences if the mushroom nugget treatments were compared.

This taste of chicken nuggets is preferred over mushroom nuggets because panellists are more familiar with the taste of chicken nuggets compared to mushroom nuggets. Apart from that, a food product is said to taste delicious because of its distinctive savoury taste.

The term for a savoury and distinctive taste is called umami (derived from Japanese). Equivalent Umami Concentration (EUC) is a quantitative parameter used to express the intensity of the umami taste in a food. It is known that the EUC value of straw mushrooms is 1181%, followed by enoki mushrooms at 139% and oyster mushrooms at 48%. The research results show that straw mushroom nuggets are the treatment with the highest favorability value, and oyster mushroom nuggets are the treatment with the lowest favorability value on taste parameters, according to the panellists.

The taste of nuggets is also related to the spices used. All nugget samples use salt, garlic, shallots, stock powder and pepper seasonings, which can enhance the taste. Therefore, the results of the data analysis show that the panellists' preferences for taste in the mushroom nugget treatment did not show a significant difference, even though the type of mushroom used as the raw material was different.

Texture

Organoleptic testing revealed that the average panellist's preference for the texture of mushroom nuggets enriched with Moringa leaves fell within the range of 5.80 to 7.20. Dunn's further test results showed that the panellists' preferences for the texture of chicken nuggets as a control were not significantly different from straw mushroom nuggets and enoki mushroom nuggets. However, chicken nuggets as a control were significantly different from oyster mushroom nuggets. No significant differences were found between mushroom types across treatments ($p > 0.05$).

This texture of chicken nuggets is preferable to mushroom nuggets because the texture of chicken meat is somewhat rough in fibre (Hidayah et al., 2021). This texture can provide a sensation of resistance when bitten so that panellists feel more comfortable when chewing.

Texture is an organoleptic assessment parameter that involves mechanical properties such as hardness. Hardness is the compressive force needed to break down a food product. Respectively, the hardness value of chicken breast meat is 56.71 N (Mehmood et al., 2019), enoki mushroom is 1766.3 gf (17.32 N), straw mushroom is 13 .75 N (Fisol et al., 2022), and oyster mushrooms of 0.2-0.4 kgf (1.96-3.92 N) (Kortei et al., 2015). It can be concluded that oyster mushrooms have the lowest hardness value compared to other mushrooms. According to the panellists, this is due to the research that shows oyster mushroom nuggets are the nugget sample with the lowest texture preference value in the neutral category. Meanwhile, the higher hardness values for enoki mushrooms and straw mushrooms indicate a liking value for the texture of the nugget in the slightly preferred category, according to the panellists.

The texture of the nugget is also influenced by the binder, which consists of cornstarch, wheat flour, and white bread. The binding agent prevents shrinkage due to cooking, increases product elasticity, and forms a dense texture. Therefore, the results of data analysis in this study show that the panellists' preferences for the texture of mushroom nuggets do not show a real difference, even though the types of mushrooms used as raw materials are different.

Effect of Mushroom Type on Nutrition of Mushroom Nugget Fortified with Moringa Leaves: Protein Content and Fat Content

The nutritional content of the nuggets was analyzed based on protein and fat content. Chicken nuggets had the highest protein content, while straw mushroom nuggets had the highest fat content. Table 3 shows the nutritional analysis results of mushroom nuggets fortified with Moringa leaves.

Table 3. Average nutrient content of mushroom nuggets fortified with Moringa leaves

	F0	**F1**	**F2**	**F3**
Protein content	20,37 ± 0,86[a]	15,51 ± 0,24[a]	14,90 ± 0,20[a]	14,93 ± 0,18[a]
Fat content	8,95 ± 0,35	8,78 ± 0,55	9,68 ± 0,32	8,96 ± 0,52

Note: F0 = chicken mushroom nuggets (control), F1 = oyster mushroom nuggets, F2 = straw mushroom nuggets, F3 = enoki mushroom nuggets. Numbers in the table followed by the same superscript letter (a) in different columns are not significantly different (p>0.05)

Protein Content

The average protein content of the nuggets, as determined by the Kjehdahl method, ranged from 14.90% to 20.37%. The treatment with the highest average protein content was oyster mushroom nuggets at 15.31%, and the treatment with the lowest average protein content was straw mushroom nuggets at 14.90%. All treatments with different types of mushrooms in nuggets had a lower average protein content than chicken nuggets as a control. Based on Dunn's further tests, it was discovered that differences in mushroom types on nugget protein levels did not make a significant difference ($p>0.05$).

It is known that the protein in raw chicken meat is 18.2% (Direktorat Jenderal Kesehatan Masyarakat, 2018). This high value is to the research results, which showed that the chicken nugget sample as a control sample had the highest average protein content of 20.37%.

Respectively, raw straw mushrooms have a protein content of 12.38% (Yuen et al., 2014), oyster mushrooms 2.75%, and enoki mushrooms 2.66% (Feeney et al., 2014). The mushroom nugget sample treatment with the highest average protein content was oyster mushroom nugget at 15.51%. A study by Dril et al. (2021) shows that the protein content in oyster mushrooms due to heat treatment is higher than when fresh. This occurs due to protein release due to damage to part of the cell wall during heating.

Apart from that, in nugget products, protein is predominantly obtained from mushrooms as a raw material and from additional ingredients such as Moringa leaf flour and eggs. It is known that every 100 g of Moringa leaf flour contains a protein content of 23.78%. As the protein content of eggs is 12.4%, according to the Direktorat Jenderal Kesehatan Masyarakat (2018), all mushroom nugget samples analyzed in this study exceeded the minimum protein content standard established by the Indonesian National Standard (SNI Nugget Protein Content = 12%).

Fat Content

As determined by Soxhlet extraction, the average fat content in nuggets was found to be within the range of 8.78% to 9.68%. The treatment with the highest average fat content was straw mushroom nuggets at 9.68%, and the treatment with the lowest average fat content was oyster mushroom nuggets at 8.78%. All nugget samples had fat content by SNI regulations (maximum fat content = 25%). Statistical analysis revealed that the

type of mushroom did not have a significant impact on the fat content of the nuggets ($p > 0.05$).

The fat content in raw enoki mushrooms is 0.28%, oyster mushrooms 0.33%, and straw mushrooms 0.77%. The fat content was significantly higher in all mushroom nugget treatments than in the raw mushroom, especially with straw mushroom nugget samples with the highest average fat content. According to Asamoa et al. (2018), the heating process does not affect the fat content of straw mushrooms.

Consequently, the augmented fat content can be attributed to the supplementary ingredients, specifically eggs. Direktorat Jenderal Kesehatan Masyarakat (2018), stated that the fat content in eggs is 10.8%. Apart from that, in the pre-frying process, some cooking oil enters the nugget and fills the previously contained water cavity. The amount of oil absorbed to soften the nuggets is the same amount as water evaporated during frying. More oil is absorbed if the nugget layer gets thicker.

A different phenomenon occurred in chicken nugget samples as a control. According to Direktorat Jenderal Kesehatan Masyarakat (2018), the fat content in chicken meat can reach 25%. In this research, the part of the chicken used was the breast. Research by Nurhayati et al. (2020) indicates that raw chicken breast has a fat content of 12.35%. Meanwhile, the fat content of the chicken nugget samples in this study was 8.95%. Therefore, the chicken nugget samples exhibited a reduction in lipid content.

Strength Level of Antioxidant Activity of Mushroom Nugget Fortified with Moringa Leaves

Based on the results of antioxidant activity testing by the DPPH method, it is known that the IC50 value of mushroom nuggets fortified with Moringa leaves ranges from 5,5 ppm to 34,547 ppm in the very active category. The nugget with the best IC50 value is the enoki mushroom nugget with an IC50 value of 5,500 ppm. The next nugget with the very active antioxidant activity category is the straw mushroom nugget with an IC50 value of 16,067, followed by oyster mushroom nuggets with an IC50 value of 34,547 ppm. Meanwhile, chicken nuggets have an IC50 value of 67,666 ppm in the active category. This shows that mushroom nuggets fortified with Moringa leaves have a better antioxidant activity category than chicken nuggets as a control.

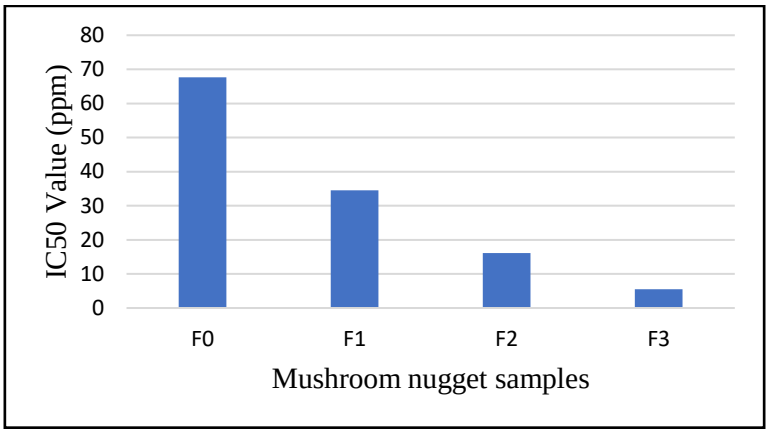

Figure 1. IC50 Value of Antioxidant Activity Analysis Results

The antioxidants in this mushroom nugget product are mainly obtained from Moringa leaves. Phenolic and flavonoids are the main antioxidant compounds in Moringa leaves. Phenolic compounds have antioxidant activity due to their ability to inactivate free radicals by binding monoxide or trioxide or decomposing hydrogen peroxide. The total phenolic compounds in Moringa leaves are around 62.56mg GAE/g (Fachriyah et al., 2020). In the group of flavonoid compounds, one of the compounds with vigorous antioxidant activity is quercetin. It is known that the quercetin in Moringa leaves is around 384.61 mg/100 g (Bhagawan et al., 2017). Quercetin's IC value of 3.5076 ppm indicates that quercetin has potent antioxidant activity (Fachriyah et al., 2020).

The nugget samples with the highest to lowest antioxidant activity were enoki mushroom nuggets, straw mushroom nuggets and oyster mushroom nuggets. Mushrooms are also known as a source of phenolic and flavonoids. Differences in antioxidant activity values in mushroom nuggets can occur due to the amount of flavonoid and phenolic compounds in each type of mushroom. The higher the levels of flavonoids and phenolics, the higher the antioxidant activity. Respectively, the flavonoid levels (mg QE/g) in enoki mushrooms, straw mushrooms and oyster mushrooms were 41.44 (Ng & Rosman, 2019), 7.29 (Boonsong et al., 2016), and 1.04 (Han et al., 2015). Then, the phenolic content (mg GAE/g) in enoki mushrooms, straw mushrooms and oyster mushrooms was 7.58, 3.16 (Khairunnisa, 2021), and 4.47 (Woldegiorgis et al., 2014). It can be concluded that enoki mushrooms have the highest amount of phenolics and flavonoids compared to other mushrooms. This aligns with research results that show the best IC50 is a sample of enoki mushroom nuggets fortified with Moringa leaves.

In chicken meat, the peptide compound carnosine is known to function as an antioxidant. Due to its physiological functions, Carnosine is a dipeptide composed of Alanine and Histidine, which is considered a bioactive food component. Chicken breast contains around 1.22mg/g carnosine (Kopec et al., 2020). IC50 of Carnosine is 9.81-16.23 ppm (Tehrani et al., 2018).

CONCLUSION

All samples of mushroom nuggets fortified with Moringa leaves were accepted by the panellists with a neutral to somewhat favourable level based on organoleptic parameters. The results of data analysis showed that control nuggets were significantly different from all nugget treatment samples with different types of mushrooms in terms of aroma, taste and texture parameters. However, the treatment of nuggets with different types of mushrooms did not show significant differences in aroma, taste and texture parameters. The differences in mushroom types on the nuggets' protein and fat content did not provide a significant difference ($p>0.05$). The IC50 value for the antioxidant activity of mushroom nuggets fortified with Moringa leaves ranges from 5,500 ppm to 34,547 ppm in the very active category.

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