

Physical, chemical, and organoleptic properties of oyster mushroom (*Pleurotus ostreatus*) meat analogue nuggets

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Abstract

Previous research has shown that meat analogue was not produced as a derivative product. The present work was conducted to produce a derivative meat analogue product (made from Indonesian local soy flour 'Anjasmoro' variety, and local lesser yam flour also known as 'gembili') with the substitution of oyster mushroom. Its purpose was to analyse the physicochemical and organoleptic properties of the nuggets produced, and ascertain the appropriate concentration of meat analogue and oyster mushroom in the product, as prescribed by the quality requirement criteria of Indonesian Standard No. 01-6683-2014 (chicken nuggets). The present work utilised a Completely Randomised Design with a single factor, including the addition of meat analogue and oyster mushroom concentration, and three times replications. The results showed that the various formulations of meat analogue and oyster mushroom affected the lightness, texture, water content, protein, fat, carbohydrate, total dietary fibre, taste preference, and aroma, but not the content, colour preference, texture, and overall. The selected formulation of the oyster-mushroom-added meat analogue nuggets was P4 (60% meat analogue:40% oyster mushroom). The nuggets had lightness, texture, water content, ash, fat, protein, carbohydrate, and total dietary fibre of 58.33, 84.13 g/3.5 mm, 37.54%, 2.11%, 3.84%, 17.87%, 38.64%, and 1.75%, respectively. The meat analogue nugget showed potential as a vegetarian fibre-rich food derived from Indonesian local plant-based ingredients.

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Introduction

In today's Indonesia, most consumers prefer instant semi-cooked food, such as nuggets, which can be made from processed meats (chicken, beef, mutton, or fish), making them comparable to the food Indonesians consume daily. They are convenient foods that are simple to cook and make at home, and have likely become Indonesians' main dish. Nuggets are created using meat restructuring technology, in which meat pieces are bound together and shaped to resemble common cuts of meat (Qin *et al.*, 2018). Due to its high protein content and other nutrients such as fats, carbohydrates, minerals, and vitamins, meats are frequently used as a raw material in nugget processing.

On the other hand, consuming processed meat products has frequently resulted in various health issues. Consumption of processed meat can increase the risk of diet-related diseases such as cardiovascular diseases and certain cancers (Guo *et al.*, 2020).

However, eliminating or partially eliminating meat from one's diet requires obtaining sufficient proteins from alternative sources instead. The so-called meat analogues can be highly beneficial, especially when starting a new diet. These products are designed to function similarly to meat and its derivative products in terms of sensory characteristics, including flavour, scent, texture, and nutritional content (Dekkers *et al.*, 2018). Meat analogues are also manufactured using protein ingredients, one of their essential product identities (Bohrer, 2019). Plant protein sources can now replace familiar animal protein sources in making meat analogues. Since plant protein-based meat analogues are low in saturated fats and sodium, cholesterol-free, and excellent source of protein, they are nearly equivalent to animal meat (Mazlan *et al.*, 2020).

Developed meat analogues are distinguished by their realistic imitation of meat structure and functionality. The significant components of meat analogues are textured vegetable proteins, which are

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fibrous matrix of vegetable protein that closely resembles the fibrillar structure of meat muscle (Caporgno *et al.*, 2020). Protein isolates are commonly used as texturising raw materials, and can be derived from soy, pea, and even fewer common sources like lupin, hemp, mung bean, and wheat gluten. It has been reported that soybean, pea, and wheat proteins are the most used building proteins for meat analogues (Sha and Xiong, 2020). Although these are inexpensive and widely available raw materials, obtaining the fibrous structure characteristic of meat proteins from soy and pea protein is a significant challenge.

Soy proteins are classified based on their protein content into three commercially available products: defatted soy flour (SF), soy protein concentrate (SPC), and soy protein isolate (SPI) (Lamaming *et al.*, 2021). Soy protein isolate (SPI) is more commonly used to develop meat analogues due to its high protein content (90%). SPI-based meat analogues have been shown to have a homogeneous structure (Chiang *et al.*, 2019), good gelling properties, and the formation of an interlaced, fibrous matrix (Gautam *et al.*, 2021). Recent research found that combining soy proteins with wheat gluten or pectin could produce a meat analogue with a wide range of fibrous structures (Schreuders *et al.*, 2019). Since the biopolymers form two separate phases, the rheological properties depend on the moisture content, the ratio of the different biopolymers, and the processing conditions (Grabowska *et al.*, 2016). Also, there were some other recent studies about the combination of SPI and gluten as ingredients to develop meat analogues (Guo *et al.*, 2020; Wi *et al.*, 2020; Chiang *et al.*, 2021), since the addition of gluten could affect the viscosity, elasticity, strength (Pietsch *et al.*, 2017), and flavour of meat analogue (Guo *et al.*, 2020).

Using shear cell technology, soy flour and other ingredients could create fibrous meat analogue structures (Geerts *et al.*, 2018). Soy flour's functionality includes its water-binding capacity and fat retention. According to Kyriakopoulou *et al.* (2021), a protein-rich meat analogue can be made from defatted soy flour with a protein content of about 50%, through a process known as fractionation, *i.e.* extraction using aqueous alcohol or acid solvent (for protein content of 70%), alkaline extraction, precipitation step in acidic pH, and neutralisation (for final protein content of 90%). The soy flour used in the present work was derived from the local

Indonesian soybean variety called 'Anjasmoro'. 'Anjasmoro' soybean was chosen due to its high protein content (about 42.1 - 46.7%) (Palupi *et al.*, 2020), and also being recommended by BALITKABI (Indonesian Research Institute of Legumes and Tubers).

In making the meat analogue, carbohydrates are added, resulting in fibrous characteristics and probably increasing texture (Lindriati *et al.*, 2020). External carbohydrates improve hardness, and enhance protein incorporation into the gel matrix in a protein-based product (Stanojevic *et al.*, 2020). The texture formed due to the interaction between carbohydrate-proteins and shear strength forms a matrix tissue. In the present work, locally obtained lesser yam flour (*Dioscorea esculenta* L., locally known as 'gembili') was used as the carbohydrate source. Lesser yam flour was chosen as an ingredient due to its bioactive compounds. The bioactive compounds found included glucomannan, diosgenin, dioscorine, and antioxidants such as phenolic and flavonoid to prevent free radicals (DPPH, hydroxyl, and superoxide hydroxy) (Murugan and Mohan, 2012; Prabowo *et al.*, 2014). Water soluble polysaccharide from lesser yam is one of water-soluble dietary fibre containing 39.49% of glucomannan (Herlina *et al.*, 2013). It has unique chemical structure, and distinctive physico-chemical properties including high water absorption, high viscosity, fermentation ability, and adsorbing or binding property for biomolecules (Schneeman and Tietzen, 1994).

There is presently no comprehensive information on derivative meat analogue products. Therefore, meat analogues produced by substituting oyster mushrooms in making nuggets can be expected to meet food requirements for healthy and safe consumption. Mushrooms have been used as meat alternatives in human diets due to their high amounts of macronutrients (proteins and fibres) and micronutrients (essential amino acids, vitamins, and minerals), as well as their small amounts of fat, sodium, and energy (Kurt and Gençcelep, 2018). Some prior research has described the incorporation of mushrooms in the manufacturing of meat analogues, such as fresh button mushrooms in a wheat gluten-based meat analogue (Ahirwar *et al.*, 2015), and oyster mushrooms in a soy protein-based meat analogue (Mazlan *et al.*, 2020).

One of the most common mushrooms used as food substitution is oyster mushroom (*Pleurotus*

ostreatus). It has high dietary fibre content, about 5.3 - 24% (Uriarte-Frias *et al.*, 2021), which aids digestion, thus suitable for the diet. This high content can increase water holding capacity, and be mutually integrated with gluten in the formation of texture. Meat analogue and oyster mushroom formulation can affect the nuggets produced. Moreover, there are also some previous studies about adding oyster mushrooms in the processing of nuggets, such as chicken nuggets from grey oyster mushroom stems and chickpea flour (Husain and Huda-Faujan, 2020), meat analogue nuggets from oyster mushroom, flaxseed, and amaranth (Nadia *et al.*, 2019), and meat analogue made from concentrated soy protein and yam (Lindriati *et al.*, 2020). The present work was specially focused on the use of Indonesian ingredients ('Anjasmoro' soy flour and lesser yam flour known as '*gembili*'). 'Anjasmoro' soy flour has been recently used with soybean milk (Dewayani *et al.*, 2020), chicken sausage with meat analogue substitution (Herlina *et al.*, 2021), white bread (Yudhistira *et al.*, 2022), soybean biscuit (Eko-Mulyo *et al.*, 2022), and snack bar (Rahmi *et al.*, 2021), while lesser yam (locally known as '*gembili*') has been recently used as farmstead bread (Ukpabi, 2010), biscuits from wild yam or lesser yam and alginate (Estiasih *et al.*, 2013), edible film (Warkoyo *et al.*, 2021), and gluten free-noodle (Ayuningtyas and Putri, 2024).

Formula combination of a derivative meat analogue ('Anjasmoro' soy flour and lesser yam '*gembili*' flour) with the substitution of oyster mushroom as meat analogue nugget has not been researched. Therefore, the present work aimed to analyse the physical, chemical, and organoleptic properties of oyster mushroom-added meat analogue nuggets, and ascertain the appropriate concentration of each element based on the quality requirement criteria of Indonesian National Standard Number 01-6683-2014.

Materials and methods

Materials

The materials used were local 'Anjasmoro' soy flour (from BALITKABI, Indonesia), lesser yam (also known as '*gembili*') flour from Indonesian local variety, oyster mushroom (*Pleurotus ostreatus*), commercial meat analogue (Rodeo, Indonesia), commercial nugget (Fiesta, Indonesia), gluten (Golden Ante, China), SPI/soy protein isolates

(Ruiqianjia, China), and seasoning (garlic, salt, and pepper powder). The present work was conducted at the Laboratory of Agricultural Product Engineering and Chemical and Biochemical Agricultural Product, Department of Agricultural Product Technology, Faculty of Agricultural Technology, University of Jember, Indonesia.

Production of 'Anjasmoro' soy flour, oyster mushroom purée, and meat analogue dough

The first step in making 'Anjasmoro' soy flour was sorting to separate malformed beans and extraneous objects. The sorted soybeans were then cleaned with water to remove impurities before being soaked for 8 h in a 3:1 water-to-soybean ratio (water changed every 2 h). This aimed to soften the soybeans, making them easier to grind. Soybeans were then boiled for 20 min to inactivate the lipoxigenase enzyme, which generates an unpleasant odour, and drained to remove any excess water. They were then dried in direct sunlight for 4 h, and then in a 60°C oven for another 24 h. Dried soybeans were ground in a grinder, and sieved through a 60-mesh sieve to obtain the 'Anjasmoro' soy flour.

The first step in making oyster mushroom purée was cleaning to remove any dirt. The fresh oyster mushroom was then chopped into little pieces, and ground with ice water in the proportion of ice water to the oyster mushroom (1.5:1). This prevented protein denaturation caused by grinding friction.

The first step in making meat analogue was to mix all the ingredients based on 100% of the analogue meat, which included gluten (50%), SPI (30%), 'Anjasmoro' soy flour (15%), and lesser yam flour (5%) using a mixer on the lowest speed for 5 min, and adding water in a water-to-meat analogue (0.5:1) ratio until homogeneous. The semi-wet meat analogue dough was ready for use in the formulation with oyster mushroom purée.

Production of oyster mushroom meat analogue nuggets

The production of nuggets began with mixing oyster mushroom purée and semi-wet meat analogue, as shown in Table 1. From the meat analogue and oyster mushroom weight, 10% garlic, 1.5% salt, and 0.5% pepper powder were added. Then, using a mixer on low speed for 15 min, everything was mixed evenly. The dough was then placed on a baking sheet (size 10 × 15 cm), and steamed for 20 min at 100°C. The baked dough was chilled in the refrigerator for 30

min at 5°C. It was then cut to 1.5 × 1.5 × 3.5 cm. Tapioca and egg whites were used to coat the nuggets. Then, they were deep fried with breadcrumbs and until golden brown.

Table 1. Formulation of oyster mushroom meat analogue nuggets.

Treatment	Meat analogue (%)	Oyster mushroom (%)
P1	90	10
P2	80	20
P3	70	30
P4	60	40
P5	50	50

Experimental design

The experimental design used was Factorial, Completely Randomized Design with a single factor of meat analogue and oyster mushroom concentration added over five treatments. The treatment was carried out three times. The observation parameters were physical properties (lightness, texture), chemical properties (moisture, ash, fat, protein, carbohydrate, total dietary fibre contents), and organoleptic properties (colour, taste, aroma, texture, overall preference).

Physical properties

Lightness

The lightness of oyster mushroom meat analogue nuggets was measured using a colour reader Konica Minolta CR-10, with the CIE Lab system with L^* (Lightness), a^* (redness), and b^* (yellowness). Before using the colour reader, standardisation was carried out using white porcelain (one set in tools), while the standard value was $L^* = 94.35$; $a^* = -5.75$; $b^* = 6.51$. The measurement was carried out three times at a different point in the surface of each treatment sample.

Texture

The texture of oyster mushroom meat analogue nuggets was measured using a Rheotex SD700. The first step was pressing the power button "ON", and set the distance button to determine the depth of the needle when penetrating the treatment sample. The depth of the needle used to measure texture was 3.5 mm. The second step was pressing button "HOLD", and the treatment sample placed on the table below the needle. The start button was pressed, and the needle punctured the treatment sample with a depth

of 3.5 mm. The scale read on tools was the texture of the treatment sample, and expressed in units of g/3.5 mm. The measurement was carried out five times at different points in each treatment sample (P1 - P5), and the scales listed were recorded as X1, X2, X3, X4, and X5 in one testing. Texture was then calculated using Eq. 1:

$$\text{Texture} = \left(\frac{g}{3.5 \text{ mm}} \right) = \frac{X_1 + X_2 + X_3 + X_4 + X_5}{5} \quad (\text{Eq. 1})$$

Scanning electron microscopy (SEM) TM 3000

The structure of oyster mushroom meat analogue nuggets was examined by scanning electron microscope (SEM) TM 3000 at high magnification. Each treatment sample (P1 - P5) were captured using SEM TM 3000 at different magnifications, and the representative was selected at 1,500× magnification. The structures of oyster mushroom meat analogue nuggets were then viewed and explained. Commercial meat analogue and commercial nugget were also captured using SEM at 1,500× magnification.

Chemical properties

The analyses of water, ash, fat, protein, and total dietary fibre contents of oyster mushroom meat analogue nuggets were performed following the method of AOAC (2005). The determination of carbohydrates content was by difference method (Winarno, 2008), and calculated through 100% deducting percentage of water, ash, fat, and protein contents.

Organoleptic test

The organoleptic test parameters used were colour, taste, aroma, texture, and overall preference. The organoleptic test used 25 semi-trained panellists who were chosen to obtain accurate data as expected to represent consumer preferences for nugget made from analogue meat - oyster mushrooms. Semi-trained panellists were panellists selected from a limited circle (knowing nugget products, often consuming, and liking nugget products). Before carrying out the organoleptic test, panellists were given a brief explanation/training to recognise the properties and characteristics of the product.

Organoleptic test of oyster mushroom meat analogue nuggets was performed using a seven-point hedonic scale (1 = really dislike; 2 = dislike; 3 = rather dislike; 4 = normally like it; 5 = rather like; 6 = like; 7 = really like) (Setyaningsih *et al.*, 2010). Every

treatment (P1 - P5) was assigned with a random three digits code on the sample during testing by the panellists.

Statistical analysis

The data from the physical and chemical properties were analysed using ANOVA at a 95% confidence level, followed by Duncan Multiple Range Test (DMRT). The organoleptic test data were processed using the chi-square method, and analysed using a descriptive method. The chosen formulation was determined by adding the weight values of the organoleptic parameters, with the highest score indicating the chosen formulation.

Results and discussion

Physical properties

The physical properties of oyster mushroom meat analogue nuggets are shown in Figures 1A - 1B. Lightness as a one-color intensity in food products is frequently used as a parameter for changing the physical and chemical properties. Colour observations were made in the present work by varying the colour lightness of each study treatment. The value of colour lightness ranged from 0 to 100; Figure 1A shows the nuggets' average lightness value. The texture of food products is an important quality parameter. Figure 1B (texture) depicts the average texture values of oyster mushroom meat analogue nuggets.

Lightness

Figure 1A (lightness) shows that the highest lightness value of the nuggets corresponded to treatment P5 (50% meat analogue:50% oyster mushroom), at 59.47, and the lowest to treatment P1 (90% meat analogue: 10% oyster mushroom), at

56.25. ANOVA at confidence interval of 95% showed that the treatment significantly affected the lightness of the nuggets produced.

Figure 1A (lightness) shows that a higher level of meat analogue used in making the nuggets resulted in a darker colour (lower value of lightness) than the other treatments, with a lower formulation of meat analogue. This result was caused by the raw material used in making the meat analogue. Gluten is white, with a lightness value of 91.23, while SPI has a white to yellow colour with a lightness value of 88.27. 'Anjasmoro' soy flour had a lightness value of 91.86, and lesser yam flour had a rather dark colour, with a lightness value of 69.35.

The formulation of meat analogue could be used in the making of nuggets, which resulted in dark colour (lower value of lightness). This result was caused by using SPI as a raw material. The use of 70% SPI caused the colour of the meat analogue to darken, with a lightness value of 56.59 ± 0.93 (Kitcharoenthawornchai and Harnsilawat, 2015).

The carbohydrate contents in 'Anjasmoro' soy and lesser yam flour can cause a Maillard reaction, a non-enzymatic browning reaction between amino compounds and reducing sugars, producing brown pigments known as melanoidins (Starowicz and Zieliński, 2019).

Furthermore, higher levels of oyster mushrooms also meant higher water levels, so the lightness of the colour was higher (brighter). This high proportion of water has many surfaces, resulting in greater light reflection with limited absorption, lowering colour intensity or making the colour appear brighter. Reflectivity is significantly related to scattering, so the soluble compounds, such as pigments, were likely scattered along with the light, and significantly reduced the colour intensity of nuggets (Mir *et al.*, 2017; Hughes *et al.*, 2020).

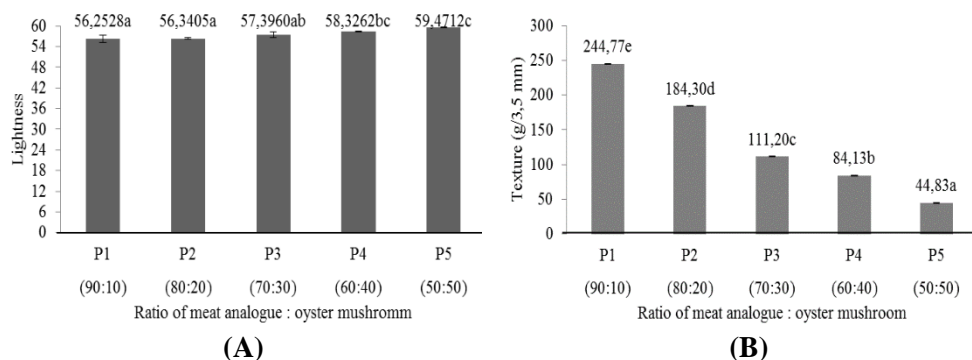


Figure 1. Average values of physical properties of oyster mushroom meat analogue nuggets: (A) lightness, and (B) texture.

Texture

Figure 1B (texture) shows the average texture values of oyster mushroom meat analogue nuggets, with the highest texture value being observed in treatment P1 (90% meat analogue:10% oyster mushroom), at 224.77 g/3.5 mm, and the lowest in treatment P5 (50% meat analogue:50% oyster mushroom), at 44.83 g/3.5 mm. The higher the texture value, the more complex the nugget texture. ANOVA at confidence interval of 95% showed that the treatment significantly affected the texture of the nuggets produced.

The amount of liquid (water) in food can affect its texture and taste. The addition of liquid, especially water, washes out nutrients. Hence, the lower the water level, the more complex the texture. The obtained Figure 1B (texture) shows inverse relation with Figure 2A.

Treatment P1 (90% meat analogue:10% oyster

mushroom) had the highest protein content, as shown in Figure 2D. The greater the protein content, the greater the viscosity. Moisturising properties, which lend directly to protein-water interaction such as solubility and viscosity (interaction between protein and water), and gelling properties, which are explained by protein-protein interactions, are among the functional properties of a protein (Goff and Guo, 2019). Increasing protein concentration means dispersed molecules are not free, and that interaction between proteins is dominant, so viscosity increases. If the interaction between proteins is strong, the three-dimensional network will be folded, and water will come out from the protein structure, causing the texture to become rigid.

The carbohydrate content of the material can also improve the nugget texture because it occurs in configuration form, including protein cross-link bonds, and interaction between proteins and

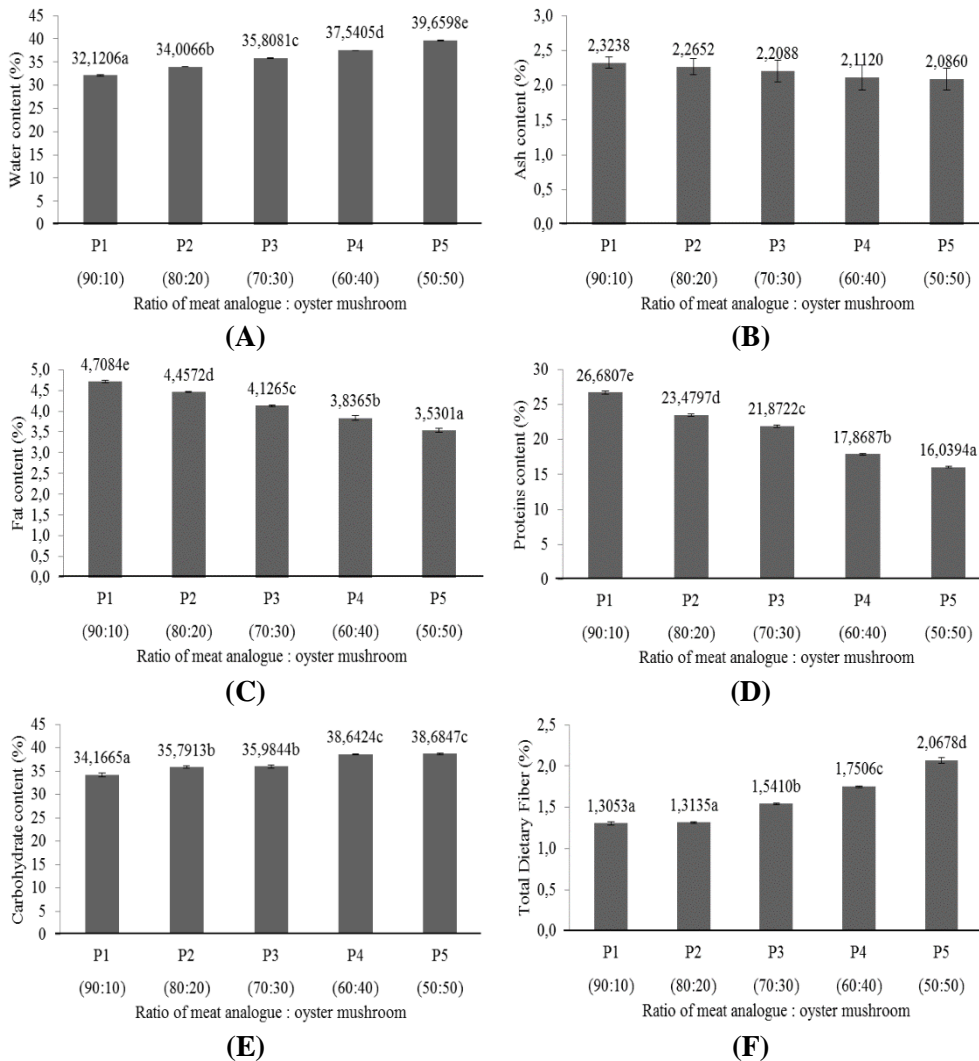


Figure 2. Chemical properties of oyster mushroom meat analogue nuggets: (A) water content, (B) ash content, (C) fat content, (D) protein content, (E) carbohydrate content, and (F) total dietary fibre content.

carbohydrates. This result can be affected by cooking and stirring. The colloids of carbohydrates or proteins are non-Newtonian fluids, so the presence of shear force affects the material viscosity (in the moving process of forming the nugget dough) (Goff and Guo, 2019). Shear speed interaction also occurs, causing the swelling of protein volume.

Interaction between proteins and carbohydrates will affect the solubility and biopolymer co-solubility, the ability to form solutions and viscous gels, viscoelasticity, and surface properties (Harijono *et al.*, 2013). That is because carbohydrates in glycoproteins act as molecular antennae. Thus, in soy-protein systems, the glycosidic parts of soy (SPI) glycoproteins were more firmly bound to the gel matrix (Ohtsuka *et al.*, 2014).

The texture and structure of food products depend on the properties of each protein and carbohydrate, and the strength interaction of its properties. It produced several possibilities, such as co-solubility (if the interaction is not accurate because two primary molecules have respective existences); incompatibility (if two polymer types repel so that they are in separate phases); and complex (if two polymers bind, causing deposits to form) (Behrouzain *et al.*, 2020).

Texture surface using SEM

Scanning electron microscopy (SEM) is a type of electron microscopy used to observe images of texture surfaces. The structure of the textures formed in meat analogue on the nuggets is one of the essential parameters, so it was used to establish the formed or not formed fibre texture. The microstructure images obtained by SEM are shown in Figures 3A - 3B of commercial product, and Figures 4A - 4E of the treatment.

The formation of protein tissues when making meat analogue occurred with the addition of 10% soy proteins. The SEM results in Figure 3A show that the commercial meat analogue fibre structure is compact, with a few hollows. In contrast, Figure 3B shows the commercial nuggets' SEM results, indicating that a fibre structure is formed with many hollows. This result was caused by the process of making commercial nuggets by pre-frying to form their texture, and allowing them to be frozen and then sold. The hollows were formed because fat globules were trapped in the broken protein matrices. Water was evaporated in the pre-frying process, and water

transfer with fat globules could not come out from the matrix.

As shown in Figure 4A, treatment P1 (90% meat analogue:10% oyster mushroom) formed more fibre structures with a few hollows than other treatments, and was very similar to the commercial meat analogue in Figure 3A. Treatment P1 (90% meat analogue:10% oyster mushroom) had higher formulation of meat analogue with high-protein material and oyster mushroom with lower water content. If there is a low water level, the protein network is formed by a compact fibre structure, producing a compact nugget structure. This result was compatible with the research conducted, which found that meat analogue SEM showed the existence of a fibre structure formed by the protein-protein network.

The higher the level of oyster mushroom used, the less compact the form of the fibre structure, with big hollows (Figure 4E). Treatment P5 (50% meat analogue:50% oyster mushroom) was used as the highest formulation of oyster mushroom and the highest water content. Using more oyster mushroom flour can make the meat analogue surface rough (Mazlan *et al.*, 2020).

Chemical properties

Water content

The chemical properties of oyster mushroom meat analogue nuggets are shown in Figures 2A - 2E. Water is an essential food component, and can affect its appearance, texture, and flavour. Based on Figure 2A (water content), the highest water content was found in treatment P5 (50% meat analogue:50% oyster mushroom) at 39.66%, and the lowest was found in treatment P1 (90% meat analogue:10% oyster mushroom) at 32.12%. ANOVA at 95% confidence interval revealed that the treatment had a significant effect on the water content of the nuggets produced. All of meat analogue nuggets meet the requirements of Indonesian National Standard No. 01-6683-2014 (maximum 50% of water content) in term of chicken nugget because Indonesia does not yet have an SNI for meat analogue nuggets.

The result from Figure 2A is inverse to Figure 2D of the protein content of oyster mushroom meat analogue nuggets. The higher the protein content, the higher the water-holding capacity (lower water content), and *vice versa*. Higher protein content in food products causes water-holding capacity to increase (lower water content) because polar groups

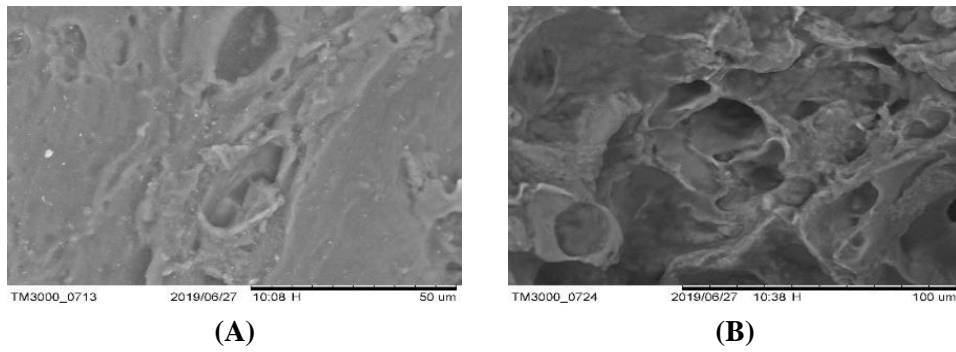


Figure 3. SEM images of (A) commercial meat analogue, and (B) commercial nuggets at 1,500× magnification.

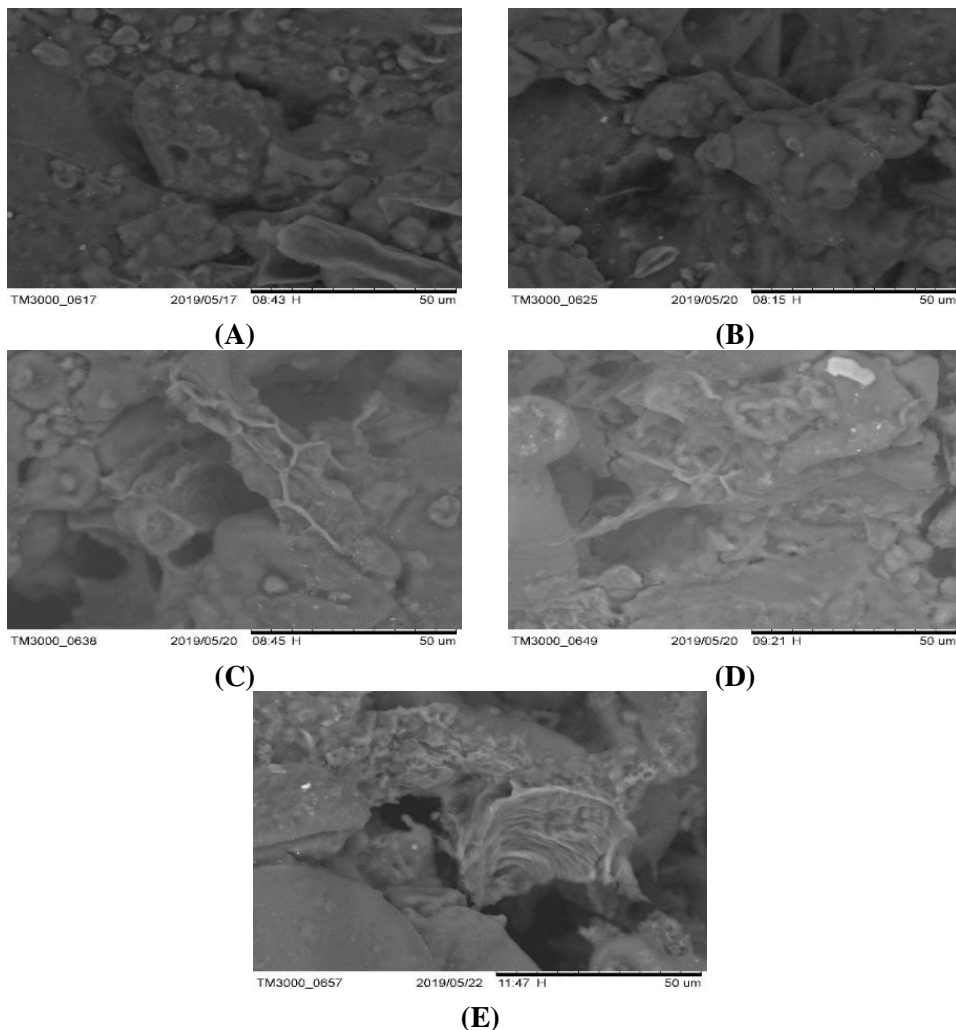


Figure 4. SEM images of oyster mushroom meat analogue nuggets with treatments (A) P1 (90:10), (B) P2 (80:20), (C) P3 (70:30), (D) P4 (60:40), and (E) P5 (50:50) at 1,500× magnification.

on proteins can lead to interaction with hydrogen ions from water (Wouters *et al.*, 2016; Wang and Xie, 2019).

The higher SPI in treatment P1 (90% meat analogue:10% oyster mushroom), the lower the water content. This result was because SPI contained hydrophilic (able to hold water) and hydrophobic (unable to hold water) amino acid groups (Jiang *et al.*,

2015; Wouters *et al.*, 2016). A higher level of gluten was used in treatment P1 (90% meat analogue:10% oyster mushroom), so the water content decreased. This result was because gluten proteins easily adsorbed a large quantity of water, which could cause amino acid chains to interact with water. Hence, the water-holding capacity was higher (Wang and Xie, 2019).

Ash content

Ash content refers to mineral elements, also known as inorganic substances. Based on Figure 2B (ash content), the highest ash content was in treatment P1 (90% meat analogue:10% oyster mushroom) at 2.32%, and the lowest in treatment P5 (50% meat analogue:50% oyster mushroom) at 2.08%. The treatment did not affect the ash content of the nuggets produced, based on ANOVA at 95% confidence interval. Since the concentration formulation range of each treatment was very similar, the treatment had no significant effect.

The ash content of the nuggets can be affected by the ingredient of each raw material. Gluten has an ash content that varies from 0.7 to 1.2% (Schopf *et al.*, 2021), SPI has 5.2% (Wulandari *et al.*, 2019), 'Anjasmoro' soy flour has 5.2%, lesser yam flour has between 0.61 and 2.21% (Asiyanbi-Hammed and Simsek, 2018), while oyster mushroom has 9.8% (Uriarte-Frias *et al.*, 2021). Based on the ingredients of the raw material used, adding higher quantities of gluten, SPI, 'Anjasmoro' soy flour, and lesser yam flour can increase the ash content of the nuggets.

Fat content

Figure 2C (fat content) shows that treatment P1 (90% meat analogue:10% oyster mushroom) has the highest fat content (4.70%), while treatment P5 (50% meat analogue:50% oyster mushroom) has the lowest (3.53%). Based on ANOVA at 95% confidence interval, the treatment significantly affected the fat content of the nuggets. All of meat analogue nuggets met the requirements of Indonesian National Standard No. 01-6683-2014 (maximum 20% of water content).

The higher fat content of the nuggets was caused by the ingredients of each raw material used. If a higher formulation of meat analogue is used, the fat content is also higher. Gluten has a fat content of 0.6 - 2.1% (Schopf *et al.*, 2021), SPI has 1.64 - 4.51%, 'Anjasmoro' soy flour has 18.3%, lesser yam flour has 0.2 - 0.4% (Asiyanbi-Hammed and Simsek, 2018), and oyster mushrooms has 1.6 - 50% (Uriarte-Frias *et al.*, 2021).

Protein content

Based on Figure 2D (proteins content), the highest protein content is in treatment P1 (90% meat analogue:10% oyster mushroom) at 26.68%, and the lowest in treatment P5 (50% meat analogue:50% oyster mushroom) at 16.03%. The treatment

significantly affected the protein content of the nuggets based on ANOVA at 95% confidence interval. All of meat analogue nuggets met the requirements of Indonesian National Standard No. 01-6683-2014 (minimum 9% of protein content).

The result from Figure 2D is inverse to Figure 2A of the protein content of oyster mushroom meat analogue nuggets. The higher the protein content, the higher the water-holding capacity (lower water content), and *vice versa*, as earlier discussed.

Carbohydrate content

Based on Figure 2E (carbohydrate content), the highest carbohydrate content is in treatment P5 (50% meat analogue:50% oyster mushroom) at 38.68%, while the lowest in treatment P1 (90% meat analogue:10% oyster mushroom) at 34.16%. ANOVA at 95% confidence interval showed that the treatment had a significant effect on the carbohydrate content of the nuggets. The nuggets' higher carbohydrate content was due to a higher formulation of oyster mushrooms, which had a carbohydrate content of 37 - 85% (Uriarte-Frias *et al.*, 2021).

Total dietary fibre content

Figure 2F shows the total dietary fibre content of the nuggets, where treatment P5 (50% meat analogue:50% oyster mushroom) has the highest total dietary fibre content at 2.06%. In comparison, treatment P1 (90% meat analogue:10% oyster mushroom) has the lowest at 1.30%. The treatment significantly affected the total dietary fibre content of the nuggets produced, based on ANOVA at 95% confidence interval.

A higher oyster mushroom formulation caused the nuggets' higher total dietary fibre content. Oyster mushroom has a dietary fibre content of 5.3 - 24% (Uriarte-Frias *et al.*, 2021). The weight of one nugget is around 20 g, while each serving of 100 g contains dietary fibre of about 2.604 - 4.064 g (Putri, 2018). Furthermore, the meat analogue nuggets were made from oyster mushroom, local 'Anjasmoro' soy flour, and local lesser yam flour '*gembili*', which could be a vegetarian fibre-rich food for consumption.

Organoleptic test

Organoleptic test parameters include colour, taste, aroma, texture, and overall preference. The oyster mushroom meat analogue nuggets for the organoleptic test were cooked nuggets that have gone through the frying process. The organoleptic

parameters of oyster mushroom meat analogue nuggets are shown in Table 2, and panellist preference level of oyster mushroom meat analogue nuggets are shown in Table 3.

Colour preference

The panellists' colour preference level were not significantly different. The highest percentage level of colour preference from the range "rather like" to "really like" was in treatment P4 (60% meat analogue:40% oyster mushroom) at 64%, and the lowest in treatment P1 (90% meat analogue:10% oyster mushroom) at 32% (Table 3).

The colour of the nuggets was the result of the raw material used, as discussed earlier for Figure 1A. Combining all the raw materials resulted in the colour of the nuggets becoming yellowish for all treatments, so the panellists could not distinguish between them.

Taste preference

Table 2 shows the panellists' taste preference for the nuggets. The formulation of meat analogue and oyster mushroom did not differ significantly at the confidence interval of 95%. The highest level

from the range of "rather like" to "really like" was in treatment P4 (60% meat analogue:40% oyster mushroom) at 68%, and the lowest in P1 (90% meat analogue:10% oyster mushroom) at 16% (Table 3).

The taste of the nuggets was influenced by the raw material used. Gluten, SPI, 'Anjasmoro' soy flour, and lesser yam flour had typical flour taste, while oyster mushroom had vegetable taste. When a higher formulation of oyster mushroom was used, and the vegetable taste was dominant, the meat analogue and oyster mushroom formulation created a similar blend taste. Therefore, the panellists could not distinguish between the tastes of each treatment.

Table 2. Organoleptic parameters of oyster mushroom meat analogue nuggets.

Parameter	Statistical Chi-squared	Table Chi-squared
Colour preference	18.45	31.41
Taste preference	28.99	31.41
Aroma preference	29.29	31.41
Texture preference	50.56	36.42
Overall preference	55.34	36.42

Table 3. Panellists' preference level of oyster mushroom meat analogue nuggets.

Preference	Level (%)	Ratio of meat analogue:oyster mushroom				
		P1 (90:10)	P2 (80:20)	P3 (70:30)	P4 (60:40)	P5 (50:50)
Colour	Rather like	20	16	20	36	24
	Like	8	28	24	20	24
	Really like	4	0	0	8	8
	Total	32	44	44	64	56
Taste	Rather like	16	12	40	48	28
	Like	0	16	12	20	20
	Really like	0	0	4	0	4
	Total	16	28	56	68	52
Aroma	Rather like	20	40	52	44	48
	Like	16	16	24	28	16
	Really like	0	4	0	0	4
	Total	36	60	76	72	68
Texture	Rather like	8	12	28	32	20
	Like	8	20	12	36	12
	Really like	0	0	12	4	8
	Total	16	32	52	72	40
Overall	Rather like	12	16	20	44	24
	Like	4	8	28	28	8
	Really like	0	0	0	0	12
	Total	16	24	48	72	44

Aroma preference

Table 2 shows the panellists' aroma preference for the nuggets. The formulation of meat analogue and oyster mushrooms did not differ significantly at the confidence interval of 95%. The highest percentage level of aroma preference from the range "rather like" to "really like" was in treatment P4 (60% meat analogue:40% oyster mushroom) at 72%, and the lowest in treatment P1 (90% meat analogue: 10% oyster mushroom) at 16% (Table 3). The aroma of the nuggets was due to the ingredients used, as earlier discussed for taste preference.

Texture preference

The panellists' texture preference levels revealed in Table 2 show that the meat analogue and oyster mushrooms formulation differ significantly at the confidence interval of 95%. The highest percentage level of texture preference was in treatment P4 (60% meat analogue:40% oyster mushroom) at 72%, and the lowest in treatment P1 (90% meat analogue: 10% oyster mushroom) at 16% (Table 3).

The texture of the nuggets was caused by the raw materials used. The results showed that the texture was similar for all treatments. Gluten is chewy when moist, and when treated mechanically, elastic dough is formed. Oyster mushrooms substitute their primary role in the texture of meat analogue nuggets. Furthermore, SPI also has functional properties to elasticity gel form (Gautam *et al.*, 2021). 'Anjasmoro' soy flour and lesser yam ('*gembili*') flour also assisted in the formation of nugget texture. Interaction between carbohydrates and proteins with shear strength formed a matrix network to improve the texture (Stanojevic *et al.*, 2020). Therefore, panellists can distinguish between the textures from different treatments.

Overall preference

Table 2 shows the panellists' overall preference levels of meat analogue and oyster mushroom formulation, which differs significantly at the confidence interval of 95%. The highest percentage level of overall preference was in treatment P4 (60% meat analogue:40% oyster mushroom) at 72%, and the lowest in treatment P1 (90% meat analogue:10% oyster mushroom) at 16% (Table 3). Since the values of overall preference were determined by the parameter values of colour, taste,

aroma, and texture preference, panellists could distinguish the nuggets' overall treatment preference.

Determination of selected treatment

The selected treatment was determined based on the organoleptic properties, and obtained from the accumulation of total weight values using the descriptive method, with the highest score values determining the selected treatment. Table 4 shows the scoring of the descriptive text of the nuggets. Meat analogue nugget with P4 formulation (60% meat analogue:40% oyster mushroom) had highest total score (3.6). The panellist chose this because of balanced composition between analogue meat:oyster mushrooms, which was indicated by a texture value of 84.13 g/3.5 mm, meaning that P4 was neither hard nor soft. This was supported by SEM image (Figure 4D), that P4 had more fibre structure with many hollows but not big hollow.

Table 4. Descriptive test scoring of oyster mushroom meat analogue nuggets.

Parameter	Weight	Ratio of meat analogue: oyster mushroom				
		P1	P2	P3	P4	P5
Colour preference	0.7	-	-	-	0.7	-
Taste preference	0.9	-	-	-	0.9	-
Aroma preference	0.7	-	-	0.7	-	-
Texture preference	1	-	-	-	1	-
Overall preference	1	-	-	-	1	-
Total		-	-	0.7	3.6	-

Conclusion

The meat analogue nuggets made from oyster mushroom (*Pleurotus ostreatus*), local 'Anjasmoro' soy flour, and local lesser yam '*gembili*' flour were tested for their physical and chemical properties. The chemical properties (water, fat, protein content) of meat analogue nuggets met the requirement of Indonesian National Standard No. 01-6683-2014. The formulation of meat analogue and oyster mushroom had a significant impact on lightness, texture, water content, protein content, fat content,

carbohydrate content, total dietary fibre content, texture preference, and overall preference. However, it had no impact on ash content, colour preference, taste preference, or aroma preference. Based on the descriptive scores of the organoleptic properties, the selected formulation was treatment P4 (60% meat analogue:40% oyster mushroom), with the highest score of 3.6. The nuggets had a lightness of 58.33; a texture of 84.13 g/35 mm; water content of 37.54%; ash content of 2.11%; fat content of 3.84%; protein content of 17.87%; carbohydrate content of 38.64%; and total dietary fibre content of 1.75%. Through the present work, the use of local commodities could be maximised for sustainable food as a vegetarian fibre-rich food in the form of meat analogue nugget.

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