The effect of rising Monascus fermented durian seed

by Ignatius Srianta

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The effect of rising Monascus fermented durian seed concentration on physicochemical and organoleptic properties of meat analog consisted of sweet potato flour and gluten

[1]Srianta, I., [1]Soejanta, B.R., [1]Natanael, J., [1,*]Ristiarini, S., [1]Nugerahani, I. and [2]Tewfik, I.

[1]*Faculty of Agricultural Technology, Widya Mandala Surabaya Catholic University, Jl. Dinoyo 42, Surabaya 62825, Indonesia*

[2]*School of Life Sciences, University of Westminster, 115 New Cavendish Street, London, W1W 6UW, UK*

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Abstract

Meat analog (MA) is an innovative, versatile food product that has a texture, colour, and flavor which resembles beef but made from vegetable ingredients. MA can be prepared by combining the flour of sweet potato with gluten. To enhance the colour of MA, some colouring agents are recommended such as Monascus Fermented Durian Seed (MFDS). This study aimed to evaluate the effect of MFDS on the physicochemical and organoleptic properties of meat analog consisting of sweet potato flour and gluten. The experimental design employed a Randomized Complete Block Design (RCBD) with one factor, namely the concentration of MFDS which consist of five levels (0%, 0.2%, 0.4%, 0.6%, and 0.8%) each replicated five times. Findings revealed that MFDS had a significant effect on colour objectively and organoleptic properties (colour preference, taste, texture), but did not have any significant effect on moisture content and texture (hardness, cohesiveness, springiness). The higher the concentration MFDS, the higher redness, colour preference, and texture preference of meat analog. Based on performed organoleptic tests, the best treatment was the addition of 0.6% MFDS which produces meat analog with moisture content of 48.01%; lightness 54.4; redness 7.4; yellowness 13.9; chroma 15.3; °hue 63.4; hardness 1992.688 g; cohesiveness 0.696; springiness 0.941; and water activity (a_w) 0.946.

1. Introduction

Meat analog is a food product that has a texture, colour, aroma, and taste that resembles red animal meat like beef, but is made from vegetable ingredients. According to Egbert and Borders (2006), the compositions of meat analog are water (50-80%), textured vegetable protein (10-25%), non-textured vegetable protein (4-20%), flavoring (3-10%), fats or oils (0-15%), binders (1-5%), and colouring agents (0-0.5%). Some literature reported that meat analogs can be made from button mushrooms (Ahirwar *et al*., 2015), red bean protein curd and winged bean flour (Nurhartadi *et al*., 2014), as well as soy flour and gluten (Putri, 2019).

According to Puspita (2014) study, meat analog was made from sweet potato flour and gluten with the proportion of gluten to sweet potato flour being 80:20. Gluten acts as a texture-forming and protein source and the addition of sweet potato flour is used as a source of fibre. The meat analog processing undergoes several stages, such as mixing dry ingredients, kneading with the addition of mushroom broth solution, freezing, thawing, twisting, and boiling (Puspita, 2014). The characteristics of the resulting meat is a chewy texture and gray-brown colour.

Due to the gray-brown colour of meat analog, it is necessary to use dyes to improve its colour. Commonly used dyes are caramel colours, malt extract, Federal Food, Drug, and Cosmetic Act (FD&C) colours (Egbert and Borders, 2006), and annatto and beetroot (Galanakis, 2019). One of the natural dyes that is used in this processing is Monascus Fermented Durian Seeds (MFDS).

Angkak is rice fermented with the fungus *Monascus purpureus* which produces colour pigments as secondary metabolites. During the fermentation process, six colour pigments are produced, namely *monascin* and *ankaflavin* which are yellow, *rubropunctatin* and *monascorubin* which are orange, *rubropunctamine* and *monascorubramine* which are red (Srianta *et al*., 2014). The high carbohydrate composition of durian seeds (33.68%) is utilized by the fungus *M. purpureus* as a carbon source (Puspitadewi *et al*., 2016). Srianta (2020)

*Corresponding author.
Email: *ristiarini@ukwms.ac.id*

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has successfully extracted and identified several pigments from MFDS, which were *monascin*, *ankaflavin*, *monascorubin*, *rubropunctation*, *monascobramine*, *rubropunctamine*, *xanthomonascin A.*, *xanthomonascin B.*, *monascopyridine A.*, *monascopyridine B.* and yellow II (Srianta, 2020).

In this study, the concentrations of MFDS used were 0%, 0.2%, 0.4%, 0.6% and 0.8%. The determination of this concentration was based on Egbert and Border (2006) that at a concentration of 0.2% and 0.4%, the subjective colour appearance was not seen, thus the study was carried out to a concentration of up to 0.8%. Therefore, this study aimed to appraise the effect of MFDS at various concentration on the physicochemical and organoleptic properties of meat analog sweet potato-gluten.

2. Materials and methods

2.1 Materials

The ingredients used in the meat analog process were MFDS, mineral water, gluten flour from a food chemical shop containing 82.5% protein (N x 6.25), 1.2% fat, 7.1% water, and 0.6% ash, sweet potato flour from a local farm (Malang, Indonesia), and mushroom seasoning powder (Herring) which contains 6.7% powdered shitake mushroom and other ingredients, including salts, MSG, starch, sugar, vegetable protein hydrolysate, and yeast extract. The meat was made by gluten flour and sweet potato flour (4:1) mixed with MFDS in 5 concentration levels (0%, 0.2%, 0.4%, 0.6%, 0.8%), and blended with 1:1 mushroom broth 13% (w/v). All the ingredients were mixed manually with hands until they became viscoelastic dough and then, freeze at -18°C for 18 hrs. The dough was thawed for 1 hr and boiled at 100°C for 15 mins. The meat analog was cut into the size of 7×4×2 cm for the analysis.

2.2 Microorganism preparation

Monascus purpureus M9 culture was used in the fermentation. It was routinely cultured on Potato Dextrose Agar (PDA) slant and preserved at 4°C. The starter culture was prepared by inoculating 7 loops of the culture scrabbed from the PDA slant, then incubated at 30°C for 10 days in an incubator (WTC-Binder). The starter culture was used for the durian fermentation.

2.3 Monascus fermented durian seed preparation

Durian seeds (Petruk variant) were obtained from the durian processing home industry. Durian seeds were boiled in a $Ca(OH)_2$ solution of 5% (w/v) for 10 mins to remove the mucus. After the seed coat was peeled off, the seeds were cut into a smaller size. Fifty grams of small size durian seed was transferred into a 250 mL Erlenmeyer, mixed thoroughly, and sterilized at 121°C for 15 mins. The durian seeds were left to cool at room temperature before being inoculated with the starter. The Erlenmeyer containing the durian seeds were incubated at 30°C for 14 days in static condition with manual shaking daily. MFDS were dried in an oven at 45°C for 24 hrs and ground into a fine powder.

2.4 Analysis methods

2.4.1 Moisture content properties

The analysis of moisture content of meat analog was carried out using the thermogravimetric method (AOAC, 1990). The wet sample of 0.5 g was dried at the temperature of 105°C for 3 hrs and followed by weighing every 30 mins until a constant weight was obtained. The test was conducted in triplicates. Moisture content was reported as:

$$\text{Moisture Content (\%)} = \frac{W_{sample}(g) - W_{dried\ sample}(g)}{W_{sample}(g)} \times 100\%$$

2.4.2 Testing colour properties

Colour testing on meat analog was carried out using a CR-20 colour reader (Konika Minolta Business Technologies Inc., Langengagen/Hannover, Germany). The results were expressed as L (lightness; 0 = black, 100 = white), a* (-a = greenness, +a = redness), b* (-b = blueness, +b = yellowness), C (chroma; 1-100), and °h (hue; 0° - 360°). The wet sample (size: 7×4×2 cm) was prepared inside the transparent plastic and measured with colour reader. The test was conducted in triplicates.

2.4.3 Testing textural properties

The wet sample (size: 7×4×2 cm per piece) was prepared before measuring textural properties. Texture Profile Analyzer (TPA) proceeded with a texture analyzer (TA-XT Plus, Stable Micro System, Surrey, UK) using a 35 mm diameter cylinder probe. The pre-test speed of 2 mm/s, the test speed of 1.5 mm/s, the post-test speed of 5 mm/s, distance target 10 mm, time 3 seconds, and the trigger force of 20 g were set up as texture analysis conditions. The calculation of hardness, springiness, and cohesiveness, were expressed according to the method of Ikhlas *et al.* (2011). The results averaged from 5 measurements.

2.4.4 Testing sensory evaluation

Sensory evaluation was carried out using the hedonic test (preferred test). The wet sample (size: 7×4×2 cm) was cut into eight pieces and fried at 150°C for 1.5 mins. Sensory evaluation was performed using fifty untrained panelists comprising of undergraduate students from Faculty of Agricultural Technology, Widya Mandala

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Table 1. Colour of meat analog with the addition of MFDS

MFDS (%)	L	a*	b*	C	Hue (°)
0	60.3 ± 0.4^{d}	2.5 ± 0.3^{a}	17.5 ± 0.2^{c}	17.3 ± 0.5^{c}	81.6 ± 0.7^{e}
0.2	57.4 ± 0.5^{c}	4.3 ± 0.3^{b}	15.3 ± 0.2^{b}	16.2 ± 0.5^{b}	74.5 ± 1.0^{d}
0.4	54.9 ± 0.6^{b}	6.0 ± 0.5^{c}	15.1 ± 0.3^{b}	15.9 ± 0.6^{ab}	68.9 ± 0.9^{c}
0.6	54.4 ± 0.4^{b}	7.3 ± 0.5^{d}	13.9 ± 0.4^{a}	15.3 ± 0.7^{a}	63.4 ± 0.7^{b}
0.8	53.1 ± 0.6^{a}	8.5 ± 0.4^{e}	13.7 ± 0.4^{a}	16.1 ± 0.5^{b}	59.3 ± 0.9^{a}

Values are presented as mean ± SD. Values with different superscript within the same column are significantly different ($p<0.05$).

Surabaya Catholic University, Surabaya, Indonesia. The panelists were asked to evaluate for colour, texture, and taste of meat analog MFDS. The scaled used were from 1 to 7 where 1 for very dislike to 7 for very like.

2.4.5 Assessing water activity

A water activity meter (Rotronic) was used to measure the water activity (a_w) of the wet sample of meat analog MFDS. Triplication of the reading was determined.

2.5 Statistical analysis tests

The experiments were analyzed using IBM SPSS Statistics 19 (IBM, Armonk, NY, USA). The data were analyzed using the analysis of variance (ANOVA) program. The confidence level was 95%. The mean comparison was performed using Duncan's multiple range tests. Differences in each treatment were considered significant at $p<0.05$.

3. Results and discussion

3.1 Moisture content

Water was one of the components that could affect the texture, appearance, taste, and shelf life of the product. The moisture content of meat analog with the addition of MFDS ranged between 48.01-49.47% (Figure 1). The moisture content of meat analog approached the moisture content of beef which is 35-73%. MFDS contained gums that could bind with water and affect the moisture content of meat analogs. Based on statistical tests, the concentration of MFDS had no significant effect on the moisture content of meat analog. This can be explained based on the low concentrations of MFDS. There was also evidenced in Liu *et al.* (2010), where beef sausage with the addition of 1.5% angkak did not show a significant difference in moisture content.

3.2 Enhanced colour properties

The colour was one of the factors of consumer acceptance of food products. Meat analog with the addition of MFDS was expected to have a colour that resembles animal meat, especially beef. Based on the results of the studies, the lightness value was obtained between 53.1 - 60.3; redness value between 2.5 – 8.5; yellowness value between 13.7 – 17.5; chroma value between 15.3 – 17.3; and hue value between 59.3 – 81.6 (Table 1). ANOVA tests showed that, the concentration of MFDS has significant effects on the values of lightness, redness, yellowness, chroma, and hue of meat analog. The lightness value of the meat analog decreased with the addition of MFDS concentration. The decrease in the value of L due to the MFDS used has a dark red colour. Hence, the higher MFDS concentration produced the darker meat analog. Instead of the lightness value, the redness value of the meat analog would increase as the intensity of the red colour produced would be higher and the yellowness value would decrease because the yellow colour was covered by red. The red colour came from the red pigment found in MFDS, namely *rubropunctamine* and *monascorubramine* (Puspitadewi *et al.*, 2016). There were other red pigments, such as *N-glucosylrubropunctamine*, *N-glutarylrubropunctamine*, and *N-glutarylmonascorubramine* and *N-glucosylmonascorubramine* (Feng *et al.*, 2012). Notwithstanding, the MFDS used in the meat analog has a high level of redness which is 16.4 while yellowness was 6.2. The colour specification of MFDS that was used in meat analog production is demonstrated in Table 2. The chroma value represented the colour intensity of the product from low to high. The chroma value of analog meat decreased with the increase of MFDS concentration. The chroma value of the meat analog was close to the chroma value of the MFDS used in the meat analog. The degree of hue indicated the colour of the product that could give different colours to another. Meat analog with the addition of MFDS was in the yellow-red colour category. The addition of higher MFDS concentration will produce analog meat with a lower hue

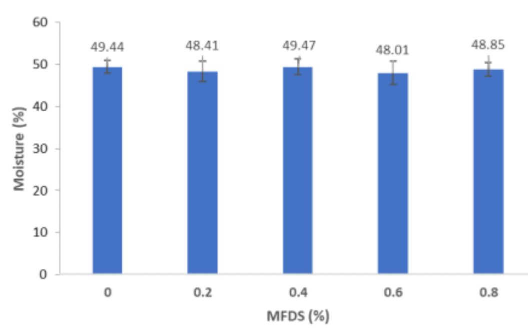

Figure 1. Moisture content of meat analog with the addition of MFDS

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value because of the red colour of the MFDS. The lower hue value will indicate that the product belongs to the red colour category, and with a higher hue value, the product will be categorized under yellow colour.

Table 2. Colour specification of MFDS

Parameter	Value
Lightness	50.3
Redness	16.4
Yellowness	6.2
Chroma	17.5
Hue	20.7

3.3 Texture properties

Based on ANOVA testing, meat analog with the addition of MFDS had no significant effect on the hardness, cohesiveness, and springiness values (Table 3). Hardness was the force required for a material to deform. The addition of MFDS caused an increase in hardness. It was due to the presence of gum in MFDS which effects the hardness level of meat analog. However, the resulting hardness level was not significantly different between treatments. Cohesiveness was the products' ability to resist deformation. The higher the cohesiveness value, the more compact the product is. The cohesiveness value of meat analog was influenced by the gum content in MFDS. Gum has hydrophilic molecules that combine with water to form a gel (Maryoto, 2008) thereof could keep meat analog from deforming. Springiness is the ability of a material to return to its original shape after being stressed. The springiness of meat analog was determined by the elasticity of the structure upon gluten formation. Gluten plays a role in forming the structural matrix of meat analog. A few concentrations of MFDS would not affect the springiness of meat analog.

3.4 Sensory evaluation

Sensory analysis was conducted to determine the level of consumer preference for the analog meat products served. The most preferrable meat analog taste is the control or without the addition of MFDS. With an increase in the addition of durian seeds, the panelist's preference score decreases (Table 4). The low score of taste preference was caused by the aftertaste of MFDS which most panelists did not like. The most likely colour preference was the addition of 0.6% MFDS. The colour of the meat analog was subjectively red-brown caused by the colour pigments in the durian seeds, *rubropunctamin* and *monascorubramin* (Puspitadewi *et al*., 2016). At the 0.8% concentration of MFDS, the score of colour preference decreased. This is due to the dark red colour of the durian seed. The highest texture preference was the addition of 0.6% MFDS. It was due to the gum content in the durian seeds that could form a gel. This caused the meat analog to be more chewy, smooth, and moist. In the treatment with the addition of 0.8% MFDS, the score of texture preference decreased compared to the addition of 0.6% MFDS which was due to the higher gum content resulting in a softer meat analog and less preferred by the panelists. It was an objective analysis on the springiness texture parameter, where the addition of 0.6% MFDS meat analog has the highest springiness value but with the addition of 0.8% MFDS concentration, the springiness value decreases.

3.5 Water activity

The best experiment from the sensory evaluation results for meat analog was the addition of 0.6% MFDS, which was further analyzed for water activity (a_w). Based on the test results, the water activity (a_w) of meat analog with the addition of 0.6% MFDS was 0.946. The water activity of meat analog with the addition of MFDS matches the water activity of beef, which was 0.98-0.99 (Lewicki *et al*., 2014). The water activity of the product was quite high, especially at 0.93-0.98. It was susceptible to being grown by gram-positive bacteria and mycotoxin -producing fungi (Naufalin, 2018). According to Merrick (2004), the expected water activity in food products for both humans and animals derived from texturized protein product (TPP) which has water activity <0.8 to prevent spoilage microbial activity. Therefore, to preserve meat analogs and extend the shelf life, meat analogs can be

Table 3. Texture of meat analog with the addition of MFDS

MFDS (%)	Hardness	Cohesiveness	Springiness
0	1596.549±149.755	0.712±0.022	0.892±0.079
0.2	1803.899±400.717	0.683±0.016	0.900±0.107
0.4	1556.075±288.020	0.720±0.013	0.855±0.120
0.6	1992.688±446.860	0.696±0.023	0.941±0.123
0.8	1743.507±368.025	0.693±0.028	0.844±0.087

Table 4. Sensory evaluation of meat analog with the addition of MFDS

MFDS (%)	Taste	Colour	Texture
0	$5.40±1.47^c$	$2.96±1.38^a$	$3.74±1.40^a$
0.2	$5.28±1.20^c$	$3.98±1.32^b$	$3.76±1.46^a$
0.4	$4.44±1.11^b$	$5.06±1.04^c$	$3.90±1.53^a$
0.6	$4.32±1.45^b$	$5.64±1.12^d$	$4.84±1.60^b$
0.8	$3.36±1.54^a$	$5.24±1.36^{cd}$	$4.50±1.39^b$

Values are presented as mean±SD. Values with different superscript within the same column are significantly different (p<0.05).

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stored in the freezer or frozen to reduce the water activity.

4. Conclusion

The concentration of MFDS significantly affected the lightness, yellowness, redness, chroma, °hue values, colour preference, taste preference, and texture preference but did not significantly affect moisture content, hardness, cohesiveness and springiness of meat analog based sweet potato flour-gluten. This optimization experiment highlighted that the best treatment of meat analog is the one with the addition 0.6% MFDS and has an a_w value of 0.946.

Conflict of interest

The authors declare no conflict of interest.

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