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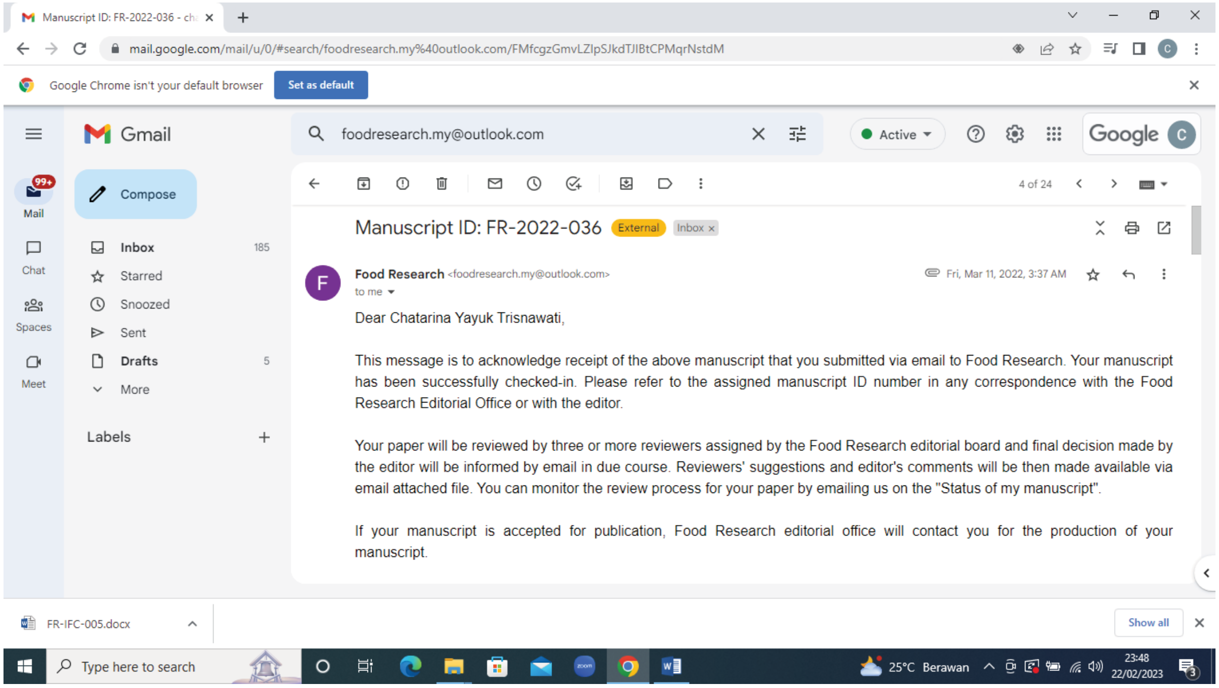
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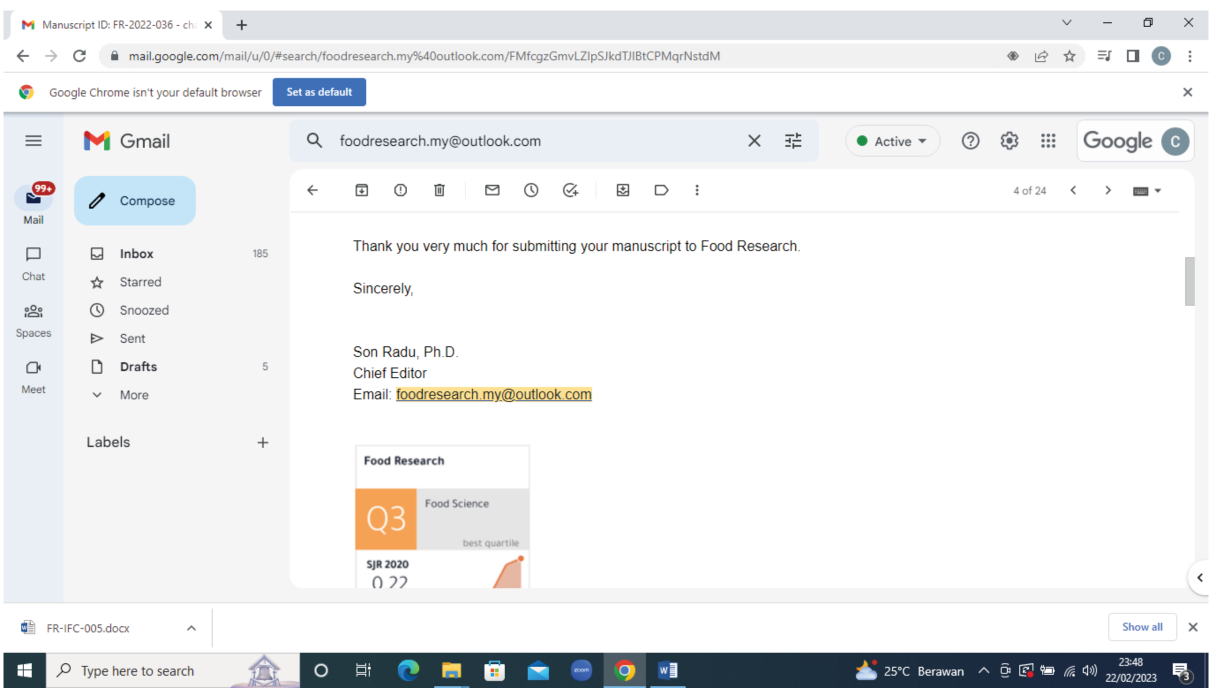
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Improving the sensory properties of bread incorporated with *Monascus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. MFDS contains monacolin K which is able reduce cholesterol while RB contains non-dietary fiber, oryzanol, and tocotrienol which can prevent hyperglycemic. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread since it contains volatile compounds with pleasant taste and aroma such as furan, furfural, and pyrazine. The aim of this study was to observe the effect of different bee pollen concentrations on the sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0%; 0.075%; 0.150%; 0.225%; 0.300%; and 0.375%. Data were analyzed by Analysis of Variance with α = 5%. The result of this research showed that different concentration of bee pollen significantly affected the sensory properties of bread incorporated with MFDS and RB. The preference score for color ranged from 4.33 to 5.45; aroma ranged from 3.35 to 5.35; taste ranged from 3.33 to 5.33; and overall acceptance ranged from 3.35 to 4.33. As bee pollen concentration increased, preference score for aroma, taste, and overall acceptance increased and preference score for color decreased. The best treatment was obtained by using 0,375% bee pollen.

Keywords: Bread, *Monascus*-fermented durian seeds, rice bran, bee pollen

1. Introduction

Bread has been a staple food for people around the centuries, from children to adult. One thing to be concerned about is the main ingredients for bread, which is wheat flour. In wheat flour production process, up to 69% of fiber content in wheat were removed leaving starch as the main component (Spanier *et al.,* 2001). During digestion process, starch are easily degraded into sugar molecules and therefore causes an increase in blood sugar level. How quickly any food causes blood sugar level increase can be translated into a number called glycemic index (Diyah *et al.,* 2016). The glycemic index value of bread is 75. Any food with a glycemic index of 70 or higher are categorized as high GI food, hence bread is a food which easily causes rise in blood sugar level. To stabilize blood sugar level, human body needs to produce insulin (Scazzina *et al.,* 2013; Konkourta *et al.,* 2017). People with diabetes have trouble in regulating level of blood sugar because their β-pancreatic cell loss which is responsible in insulin production loss its function (Gupta *et al.,* 2015).

To produce bread which is suitable for diabetic person, incorporation of bioactive compound with anti-diabetic properties is very important. One way to solve this problem is by adding functional ingredients like rice bran and *Monascus*-fermented durian seeds (Trisnawati *et al.,* 2019). Rice bran contains γ-orizanol, γ-tocotrienol and non-dietary fiber which help to regulate blood sugar level and insulin secretion (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). Nugerahani *et al.* (2017) also reported that phenolic compound and monascin pigments in *Monascus*-fermented durian seeds can help to reduce blood sugar level. *Monascus*-fermented durian seeds also contains monacolin-K which helps to reduce cholesterol production by acting as an inhibitor of hydroxymethylglutaryl-CoA (HMG-CoA) reductase (Faroukh and Baumgärtel, 2019).

Incorporation of *Monascus*-fermented durian seeds and rice bran however reduce the preference score for aroma and taste of bread incorporated with *Monascus*-fermented durian seeds and rice bran as reported by Trisnawati *et al.* (2019). Rice bran contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). In the fermentation process of *Monascus*-fermented durian seeds, secondary metabolite such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from rice bran and *Monascus*-fermented durian seeds are by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and ketons (Neto *et al.,* 2017). Incorporation of bee pollen also brings to more furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity flavor (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the sensory properties of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

2. Materials and methods

2.1 Materials

Materials that used for making bread incorporated with *Monascus*-fermented durian seeds and rice bran were “Cakra Kembar” high-protein wheat flour, “dr.Liem” rice bran flour, “Fermipan” instant dry yeast, “Dancow” instant full cream milk powder, “Gulaku” granulated sugar, “Bakerine” bread improver, “Cap Kapal” table salt, “Aqua” mineral water, “Blueband” margarine which were purchased from local distributor, “Mirah Delima” multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also *Monascus*-fermented durian seeds powder which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that used for making *Monascus*-fermented durian seeds were Petruk durian seeds, pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, aquadest and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Process of making bread incorporated with *Monascus*-fermented durian seeds and rice bran consisted of mixing (mixer: “Philips) high-protein wheat flour, rice bran flour, granulated sugar, instant dry yeast, bread improver, instant full cream milk powder and bee pollen for 1 minute. After that, water was added and mixing process continued until 20 minutes. Margarine and table salt then added and mixing process continued until minutes. The dough was rested at 26^{o}C for 30 minutes then shaped into a loaf and proofed at 26^{o}C for 90 minutes. After that, it was baked using oven (Maksindo RFL-12C) at 180^{o}C for 30 minutes then cooled at 26^{o}C for 60 minutes (Koeswanto, 2019). Table 1 shows the composition for making bread incorporated with *Monascus*-fermented durian seeds and rice bran and indicate the incorporation of bee pollen at levels control (B_0), 0.075% (B_1), 0.150% (B_2), 0.225% (B_3), 0.300% (B_4) and 0.375% (B_5)

2.3 Monascus-fermented durian seeds preparation

Durian seeds were sorted and then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90°C for 10 minutes with durian seeds : 5% $Ca(OH)_2$ solution ratio was 1:1 (w/v). The durian seeds were removed from the $Ca(OH)_2$ solution and then were washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm and were dried at 45°C for 40 minutes. Durian seeds were scaled into 50 grams. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 minutes and were cooled down at 26°C for 30 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter and were put into fermentation at 30$\pm$1°C for 14 days. The result of the fermentation were dried at 45°C for 24 hour. The dried *Monascus*-fermented durian seed was grinded and sifted to get *Monascus*-fermented durian seeds powder (Puspitadewi *et al.,* 2016).

2.4 Sensory evaluation

Sensory evaluation was carried out at quality control and sensory evaluation laboratory at Department of Food Technology, Faculty of Agricultural Technology, Widya Mandala Surabaya Catholic University. Private booths for each panelist were prepared. Mineral water was provided between samples to clean panelist's tastebud. 50 untrained panelists which consisted of undergraduate students of food technology department were asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm x 5 cm x 1 cm. There tested parameters consisted of preference score for color, aroma, taste and overall acceptance.

2.5 Moisture content

Moisture content analysis was done to support the data from the sensory evaluation Moisture content analysis was carried out using thermogravimetric method according to (AOAC 925.10). Moisture content was calculated using the formula of:

$$Moisture\ content\ (\%) = \frac{sample\ weight\ (g)}{sample\ weight\ (g) - weight\ loss\ (g)} \times 100\%$$

2.6 Color test

Color analysis was done to support the data from the sensory evaluation. Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated with *Monascus*-fermented durian seeds and rice bran with color reader (Minolta CR-10 Chroma Meter).

2.7 Statistical analysis

Statistical analysis of data for effects of different bee pollen concentration on bread incorporated with *Monascus*-fermented durian seed and rice bran was performed by one-way analysis of variance (ANOVA) and SPSS 17.0 for Windows. Mean difference was analyzed with Duncan's Multiple Range Test (DMRT) at $p \leq 0{,}05$. The optimum bee pollen concentration was determined using spider-web test on Microsoft Excel 2013. The analyzed parameters comprised of preference score for color, aroma, taste and overall acceptance.

3. Results and discussion

Sensory evaluation were carried using hedonic test method by involving 50 untrained panelist. The result of the test is showed in Table 2.

As shown in Table 2, difference in bee pollen concentration significantly affect the preference score for color, aroma, taste and overall acceptance ($P > 0.05$). The preference score for color decreased while there was an increased on aroma, taste and overall acceptance. Those difference were affected by chemical composition of bee pollen.

Bee pollen contains β-carotene which produce yellow pigment. Bee pollen also contains protein (22.7%); fructose and glucose (25.7%) and sucrose (3.7%) (Komosinska—Vassev *et al.,* 2015). The sugar in bee pollen carried free carbonyl groups and the protein carried free amine groups which then underwent Maillard reaction when exposed to high temperature during baking process and resulted in brown pigment called melanoidin. Higher bee pollen concentration resulted in more yellow and darker crumb. This statement is supported by data collected from color analysis with color reader which is showed in Table 3.

According to Table 3., there was an increase of lightness, yellowness and redness index. This data showed that higher bee pollen concentration resulted in bread with more yellowish and darker crumb. The color of the resulting bread therefore was different from what the panelists had perceived in terms of bread color, therefore lowered the preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran as the bee pollen concentration increased.

As seen in Table 2, increased in bee pollen concentration also increased the panelist preference in terms of aroma. Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone the rancid aroma, which derived from the rice bran.

Preference score for taste also increased as bee pollen concentration increased. Higher bee pollen concentration meant there were more sugar and protein in the bread dough which led into more Maillard reaction products such as furfural (caramel), 2-acetylfuran (cinnamon), furfural (caramel) and 2-pentylfuran (fruits) (Conte *et al.,* 2020). These compounds helped in covering the astringent taste which came from *Monascus*-fermented durian seeds and cardboard taste which came from the rice bran; thus higher concentration of bee pollen were preferred by the panelist. Preference score for aroma was also influenced by moisture content. Data of moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran with each bee pollen concentration is shown in Table 3.

In Table 4., it is showed that higher bee pollen concentration caused an increase in moisture content of the bread incorporated with *Monascus*-fermented durian seeds and rice bran, therefore bread with higher moisture content had higher score of aroma and taste preference. Some of volatile compounds were water-soluble and as water evaporate during heat process, there will be loss of these compounds (Raquel and Guin, 2018). Bee pollen contains protein (22.7%); fructose and glucose (25.7%) and sucrose (3.7%) (Komosinska—Vassev *et al.,* 2015), which were able to bind with water molecules; therefore reduced the water evaporation and loss of water-soluble volatile compounds in the bread dough during baking process.

Bread incorporated with *Monascus*-fermented durian seeds and rice bran achieved higher overall acceptance score as the bee pollen concentration increased as seen in Table 2. This result showed that panelist preferred bread incorporated with *Monascus*-fermented durian seeds and rice bran with higher bee pollen concentration since the taste and aroma was better, even though the color was less favorable.

The spider web test showed that the best treatments was obtained incorporation of 0.375% bee pollen into bread incorporated with *Monascus*-fermented durian seeds and rice bran. According to Figure 1., it can be seen that the preference score of color, aroma, taste and overall acceptance of 0.375% bee pollen formed the largest quadrilateral. Therefore, 0.375% bee pollen is the best concentration among the other treatments.

This research, however, is subject to limitation. Textural properties was not included in the sensorial evaluation since this research was more focused on improving the taste and aroma of the bread incorporated with *Monascus*-fermented durian seeds and rice bran. This research showed that bread with 0.375% had the highest preference score of aroma and taste. Hence, it can be suggested that research regarding textural improvement of bread incorporated with *Monascus*-fermented durian seeds and rice bran with 0.375% bee pollen to be conducted in the future. It's suggested to add hydrocolloid to improve this bread in terms of textural properties by adding hydrocolloid. According to Bourekoua *et al.* (2018) addition of starch or hydrocolloid can improve the textural quality of gluten-free bread.

4. Conclusion

Different concentration of bee pollen had significantly affected the preference score for color, aroma, taste and overall acceptance of bread incorporated with *Monascus*-fermented durian seeds and rice bran. As bee pollen concentration increased, moisture content, redness, yellowness, chroma and hue increased; lightness decreased; preference score for color decreased while the preference score for aroma, taste, and overall acceptance increased. The best treatment is 0.375% bee pollen.

This research provided new insight into the field of bakery products which are supplemented with ingredients derived from food waste such as rice bran and durian seeds. Through this research, it can be found out that addition of bee pollen helps to improve the taste and aroma of bread with food waste derived ingredients. Researcher may also consider addition of bee pollen to improve the taste and aroma of other bakery products which contains food waste derived ingredients.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Ingredients/g	B	B_1	B_2	B_3	B_4	B_5
High-protein wheat flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

Table 2. Effect of bee pollen concentration on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Sensory evaluation	B	B_1	B_2	B_3	B_4	B_5
Color	5.45 ± 1.20^{d}	5.40 ± 0.93^{d}	5.05 ± 1.20^{cd}	4.85 ± 0.98^{bc}	4.55 ± 1.15^{ab}	4.33 ± 1.54^{a}
Aroma	3.35 ± 0.92^{a}	4.43 ± 1.36^{b}	4.83 ± 1.01^{b}	4.53 ± 1.22^{b}	4.73 ± 1.26^{b}	5.35 ± 0.95^{c}
Taste	3.33 ± 0.92^{a}	3.38 ± 0.90^{a}	4.10 ± 1.01^{b}	4.48 ± 1.26^{b}	4.93 ± 1.23^{c}	5.33 ± 0.76^{d}
Overall acceptance	3.35 ± 0.77^{a}	4.33 ± 1.05^{b}	4.80 ± 1.18^{c}	4.93 ± 1.21^{cd}	5.05 ± 1.20^{cd}	5.33 ± 0.97^{d}

Values are presented as mean$\pm$SD (n = 50 for each group). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 3. Effect of bee pollen concentration on color bread incorporated with *Monascus*-fermented durian seeds and rice bran

Color evaluation	B	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2±0.25^d	67.6±0.39^c	66.8±0.99^b	66.5±0.20^b	65.7±0.55^a	65.3±0.17^a
Redness (a*)	3.2±0.13^a	3.2±0.10^a	3.3±0.18^a	3.4±0.13^b	3.6±0.10^c	3.8±0.03^d
Yellowness (b*)	15.9±0.13^a	17.4±0.28^b	18.2±0.22^c	18.6±0.45^c	19.5±0.45^d	20.1±0.32^e
Chroma (C)	16.2±0.15^a	17.7±0.28^b	18.5±0.22^c	18.9±0.45^c	19.8±0.46^d	20.5±0.33^e
Hue (^{o}H)	78.7±0.43^a	79.6±0.48^b	79.8±0.53^b	79.7±0.38^b	79.5±0.15^b	79.2±0.16^b

Values are presented as mean±SD (n = 4 for each group). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 4. Effect of bee pollen concentration on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee Pollen (%w/w)	Moisture Content (%)
0	40.29 ± 0.11^{a}
0.075	44.20 ± 0.07^{b}
0.150	47.34 ± 0.15^{c}
0.225	51.35 ± 0.16^{d}
0.300	54.23 ± 0.15^{e}
0.375	59.25 ± 0.16^{f}

Values are presented as mean$\pm$SD (n = 4 for each group). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05)

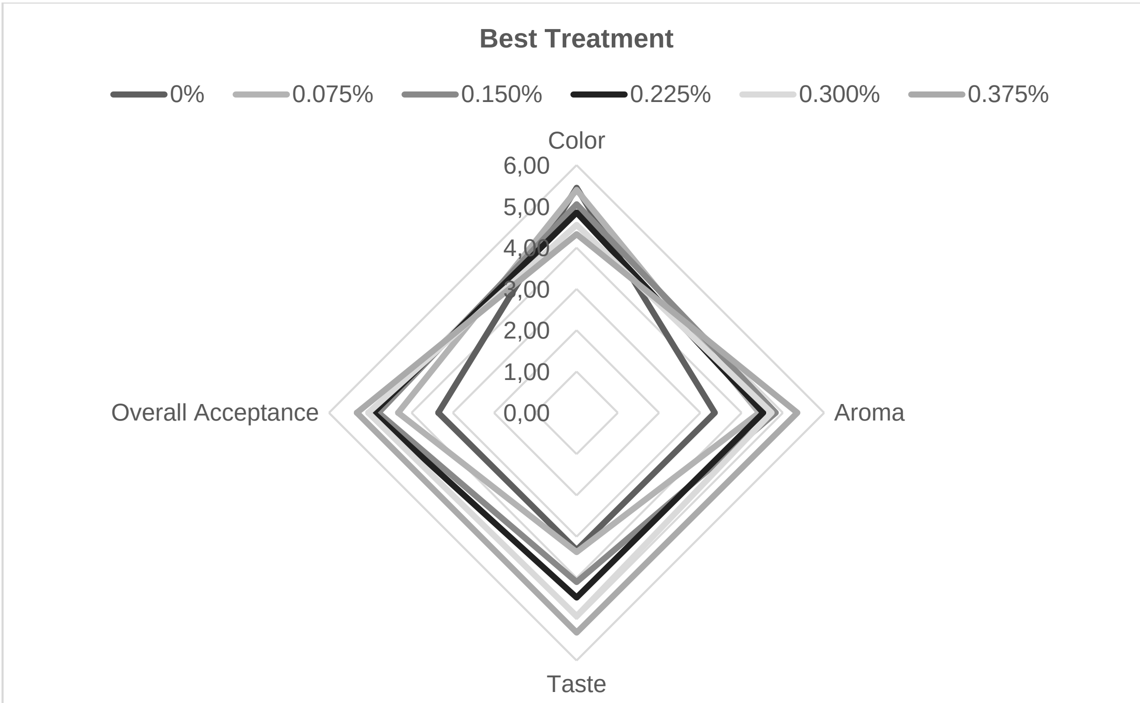

Figure 1. Spider web test result

2.

Bukti konfirmasi review dan hasil review dari 2 Reviewer

(17 April 2022)

Manuscript ID: FR-2022-036

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Improving the sensory properties of bread incorporated with *Monascus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. MFDS contains monacolin K which is able reduce cholesterol while RB contains non-dietary fiber, oryzanol, and tocotrienol which can prevent hyperglycemic. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread since it contains volatile compounds with pleasant taste and aroma such as furan, furfural, and pyrazine. The aim of this study was to observe the effect of different bee pollen concentrations on the sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0%; 0.075%; 0.150%; 0.225%; 0.300%; and 0.375%. Data were analyzed by Analysis of Variance with α = 5%. The result of this research showed that different concentration of bee pollen significantly affected the sensory properties of bread incorporated with MFDS and RB. The preference score for color ranged from 4.33 to 5.45; aroma ranged from 3.35 to 5.35; taste ranged from 3.33 to 5.33; and overall acceptance ranged from 3.35 to 4.33. As bee pollen concentration increased, preference score for aroma, taste, and overall acceptance increased and preference score for color decreased. The best treatment was obtained by using 0,375% bee pollen.

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Keywords: Bread, *Monascus*-fermented durian seeds, rice bran, bee pollen

1. Introduction

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Bread has been a staple food for people around the centuries, from children to adult. One thing to be concerned about is the main ingredients for bread, which is wheat flour. In wheat flour production process, up to 69% of fiber content in wheat were removed leaving starch as the main component (Spanier *et al.,* 2001). During digestion process, starch are easily degraded into sugar molecules and therefore causes an increase in blood sugar level. How quickly any food causes blood sugar level increase can be translated into a number called glycemic index (Diyah *et al.,* 2016). The glycemic index value of bread is 75. Any food with a glycemic index of 70 or higher are categorized as high GI food, hence bread is a food which easily causes rise in blood sugar level. To stabilize blood sugar level, human body needs to produce insulin (Scazzina *et al.,* 2013; Konkourta *et al.,* 2017). People with diabetes have trouble in regulating level of blood sugar because their β-pancreatic cell loss which is responsible in insulin production loss its function (Gupta *et al.,* 2015).

To produce bread which is suitable for diabetic person, incorporation of bioactive compound with anti-diabetic properties is very important. One way to solve this problem is by adding functional ingredients like rice bran and *Monascus*-fermented durian seeds (Trisnawati *et al.,* 2019). Rice bran contains γ-orizanol, γ-tocotrienol and non-dietary fiber which help to regulate blood sugar level and insulin secretion (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). Nugerahani *et al.* (2017) also reported that phenolic compound and monascin pigments in *Monascus*-fermented durian seeds can help to reduce blood sugar level. *Monascus*-fermented durian seeds also contains monacolin-K which helps to reduce cholesterol production by acting as an inhibitor of hydroxymethylglutaryl-CoA (HMG-CoA) reductase (Faroukh and Baumgärtel, 2019).

Incorporation of *Monascus*-fermented durian seeds and rice bran however reduce the preference score for aroma and taste of bread incorporated with *Monascus*-fermented durian seeds and rice bran as reported by Trisnawati *et al.* (2019). Rice bran contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and

Samuel, 2009). In the fermentation process of *Monascus*-fermented durian seeds, secondary metabolite such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from rice bran and *Monascus*-fermented durian seeds are by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and ketons (Neto *et al.,* 2017). Incorporation of bee pollen also brings to more furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity flavor (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the sensory properties of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

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2. Materials and methods

2.1 Materials

Materials that used for making bread incorporated with *Monascus*-fermented durian seeds and rice bran were "Cakra Kembar" high-protein wheat flour, "dr.Liem" rice bran flour, "Fermipan" instant dry yeast, "Dancow" instant full cream milk powder, "Gulaku" granulated sugar, "Bakerine" bread improver, "Cap Kapal" table salt, "Aqua" mineral water, "Blueband" margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also *Monascus*-fermented durian seeds powder which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that used for making *Monascus*-fermented durian seeds were Petruk durian seeds, pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, aquadest and potato dextrose agar (Merck 1.10130.0500).

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2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Process of making bread incorporated with *Monascus*-fermented durian seeds and rice bran consisted of mixing (mixer: "Philips) high-protein wheat flour, rice bran flour, granulated sugar, instant dry yeast, bread improver, instant full cream milk powder and bee pollen for 1 minute. After that, water was added and mixing process continued until 20 minutes. Margarine and table salt then added and mixing process continued until minutes. The dough was rested at 26^{o}C for 30 minutes then shaped into a loaf and proofed at 26^{o}C for 90 minutes. After that, it was baked using oven (Maksindo RFL-12C) at 180^{o}C for 30 minutes then cooled at 26^{o}C for 60 minutes (Koeswanto, 2019). Table 1 shows the composition for making bread incorporated with *Monascus*-fermented durian seeds and rice bran and indicate the incorporation of bee pollen at levels control (B_0), 0.075% (B_1), 0.150% (B_2), 0.225% (B_3), 0.300% (B_4) and 0.375% (B_5)

Commented [A6]: How high? Write the protein content

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Commented [A8]: Please add the method you use for making bread (reference), since the mixing process you use is bit longer than regular bread dough

2.3 Monascus-fermented durian seeds preparation

Durian seeds were sorted and then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90^{o}C for 10 minutes with durian seeds : 5% $Ca(OH)_2$ solution ratio was 1:1 (w/v). The durian seeds were removed from the $Ca(OH)_2$ solution and then were washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm and were dried at 45^{o}C for 40 minutes. Durian seeds were scaled into 50 grams. After that, durian seeds were sterilized at 121^{o}C, 15 lbs/inch^2 for 10 minutes and were cooled down at 26^{o}C for 30 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter and were put into fermentation at 30$\pm$1^{o}C for 14 days. The result of the fermentation were dried at 45^{o}C for 24 hour. The dried *Monascus*-fermented durian seed was grinded and sifted to get *Monascus*-fermented durian seeds powder (Puspitadewi *et al.,* 2016).

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2.4 Sensory evaluation

Sensory evaluation was carried out at quality control and sensory evaluation laboratory at Department of Food Technology, Faculty of Agricultural Technology, Widya Mandala Surabaya Catholic

University. Private booths for each panelist were prepared. Mineral water was provided between samples to clean panelist's tastebud. 50 untrained panelists which consisted of undergraduate students of food technology department were asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm x 5 cm x 1 cm. There tested parameters consisted of preference score for color, aroma, taste and overall acceptance.

2.5 Moisture content

Moisture content analysis was done to support the data from the sensory evaluation Moisture content analysis was carried out using thermogravimetric method according to (AOAC 925.10). Moisture content was calculated using the formula of:

$$Moisture\ content\ (\%) = \frac{sample\ weight\ (g)}{sample\ weight\ (g) - weight\ loss\ (g)} \times 100\%$$

2.6 Color test

Color analysis was done to support the data from the sensory evaluation. Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated with *Monascus*-fermented durian seeds and rice bran with color reader (Minolta CR-10 Chroma Meter).

2.7 Statistical analysis

Statistical analysis of data for effects of different bee pollen concentration on bread incorporated with *Monascus*-fermented durian seed and rice bran was performed by one-way analysis of variance (ANOVA) and SPSS 17.0 for Windows. Mean difference was analyzed with Duncan's Multiple Range Test (DMRT) at $p \leq 0,05$. The optimum bee pollen concentration was determined using spider-web test on

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Microsoft Excel 2013. The analyzed parameters comprised of preference score for color, aroma, taste and overall acceptance.

3. Results and discussion

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Sensory ~~evaluation were carried using hedonic test method by involving 50 untrained panelist~~. ~~The~~ result ~~of the test~~ is showed in Table 2.

~~As shown in Table~~ 2, difference in bee pollen concentration significantly affect the preference score for color, aroma, taste and overall acceptance ($P > 0.05$). The preference score for color decreased while there was an increased on aroma, taste and overall acceptance. Those difference were affected by chemical composition of bee pollen.

Bee pollen contains β-carotene which produce yellow pigment. Bee pollen also contains protein (22.7%); fructose and glucose (25.7%) and sucrose (3.7%) (Komosinska—Vassev *et al.,* 2015). The sugar in bee pollen carried free carbonyl groups and the protein carried free amine groups which then underwent Maillard reaction when exposed to high temperature during baking process and resulted in brown pigment called melanoidin. Higher bee pollen concentration resulted in more yellow and darker crumb. This statement is supported by data collected from color analysis with color reader which is showed in Table 3.

According to Table 3., there was an increase of lightness, yellowness and redness index. This data showed that higher bee pollen concentration resulted in bread with more yellowish and darker crumb. The color of the resulting bread therefore was different from what the panelists had perceived in terms of bread color, therefore lowered the preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran as the bee pollen concentration increased.

As seen in Table 2, increased in bee pollen concentration also increased the panelist preference in terms of aroma. Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone the rancid aroma, which derived from the rice bran.

Preference score for taste also increased as bee pollen concentration increased. Higher bee pollen concentration meant there were more sugar and protein in the bread dough which led into more Maillard reaction products such as furfural (caramel), 2-acetylfuran (cinnamon), furfural (caramel) and 2-pentylfuran (fruits) (Conte *et al.,* 2020). These compounds helped in covering the astringent taste which came from *Monascus*-fermented durian seeds and cardboard taste which came from the rice bran; thus higher concentration of bee pollen were preferred by the panelist. Preference score for aroma was also influenced by moisture content. Data of moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran with each bee pollen concentration is shown in Table 3.

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In Table 4., it is showed that higher bee pollen concentration caused an increase in moisture content of the bread incorporated with *Monascus*-fermented durian seeds and rice bran, therefore bread with higher moisture content had higher score of aroma and taste preference. Some of volatile compounds were water-soluble and as water evaporate during heat process, there will be loss of these compounds (Raquel and Guin, 2018). Bee pollen contains protein (22.7%); fructose and glucose (25.7%) and sucrose (3.7%) (Komosinska—Vassev *et al.,* 2015), which were able to bind with water molecules; therefore reduced the water evaporation and loss of water-soluble volatile compounds in the bread dough during baking process.

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Bread incorporated with *Monascus*-fermented durian seeds and rice bran achieved higher overall acceptance score as the bee pollen concentration increased as seen in Table 2. This result showed that panelist preferred bread incorporated with *Monascus*-fermented durian seeds and rice bran with higher bee pollen concentration since the taste and aroma was better, even though the color was less favorable.

The spider web test showed that the best treatments was obtained incorporation of 0.375% bee pollen into bread incorporated with *Monascus*-fermented durian seeds and rice bran. According to Figure 1., it can be seen that the preference score of color, aroma, taste and overall acceptance of 0.375% bee pollen formed the largest quadrilateral. Therefore, 0.375% bee pollen is the best concentration among the other treatments.

This research, however, is subject to limitation. Textural properties was not included in the sensorial evaluation since this research was more focused on improving the taste and aroma of the bread incorporated with *Monascus*-fermented durian seeds and rice bran. This research showed that bread with 0.375% had the highest preference score of aroma and taste. Hence, it can be suggested that research regarding textural improvement of bread incorporated with *Monascus*-fermented durian seeds and rice bran with 0.375% bee pollen to be conducted in the future. It's suggested to add hydrocolloid to improve this bread in terms of textural properties by adding hydrocolloid. According to Bourekoua *et al.* (2018) addition of starch or hydrocolloid can improve the textural quality of gluten-free bread.

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4. Conclusion

Different concentration of bee pollen had significantly affected the preference score for color, aroma, taste and overall acceptance of bread incorporated with *Monascus*-fermented durian seeds and rice bran. As bee pollen concentration increased, moisture content, redness, yellowness, chroma and hue increased; lightness decreased; preference score for color decreased while the preference score for aroma, taste, and overall acceptance increased. The best treatment is 0.375% bee pollen.

This research provided new insight into the field of bakery products which are supplemented with ingredients derived from food waste such as rice bran and durian seeds. Through this research, it can be found out that addition of bee pollen helps to improve the taste and aroma of bread with food waste derived ingredients. Researcher may also consider addition of bee pollen to improve the taste and aroma of other bakery products which contains food waste derived ingredients.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Ingredients/g	B	B_1	B_2	B_3	B_4	B_5
High-protein wheat flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

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Table 2. Effect of bee pollen concentration on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Sensory evaluation	B	B_1	B_2	B_3	B_4	B_5
Color	5.45 ± 1.20^{d}	5.40 ± 0.93^{d}	5.05 ± 1.20^{cd}	4.85 ± 0.98^{bc}	4.55 ± 1.15^{ab}	4.33 ± 1.54^{a}
Aroma	3.35 ± 0.92^{a}	4.43 ± 1.36^{b}	4.83 ± 1.01^{b}	4.53 ± 1.22^{b}	4.73 ± 1.26^{b}	5.35 ± 0.95^{c}
Taste	3.33 ± 0.92^{a}	3.38 ± 0.90^{a}	4.10 ± 1.01^{b}	4.48 ± 1.26^{b}	4.93 ± 1.23^{c}	5.33 ± 0.76^{d}
Overall acceptance	3.35 ± 0.77^{a}	4.33 ± 1.05^{b}	4.80 ± 1.18^{c}	4.93 ± 1.21^{cd}	5.05 ± 1.20^{cd}	5.33 ± 0.97^{d}

Values are presented as mean$\pm$SD (n = 50 for each group). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 3. Effect of bee pollen concentration on color bread incorporated with *Monascus*-fermented durian seeds and rice bran

Color evaluation	B	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2 ± 0.25^{d}	67.6 ± 0.39^{c}	66.8 ± 0.99^{b}	66.5 ± 0.20^{b}	65.7 ± 0.55^{a}	65.3 ± 0.17^{a}
Redness (a*)	3.2 ± 0.13^{a}	3.2 ± 0.10^{a}	3.3 ± 0.18^{a}	3.4 ± 0.13^{b}	3.6 ± 0.10^{c}	3.8 ± 0.03^{d}
Yellowness (b*)	15.9 ± 0.13^{a}	17.4 ± 0.28^{b}	18.2 ± 0.22^{c}	18.6 ± 0.45^{c}	19.5 ± 0.45^{d}	20.1 ± 0.32^{e}
Chroma (C)	16.2 ± 0.15^{a}	17.7 ± 0.28^{b}	18.5 ± 0.22^{c}	18.9 ± 0.45^{c}	19.8 ± 0.46^{d}	20.5 ± 0.33^{e}
Hue (°H)	78.7 ± 0.43^{a}	79.6 ± 0.48^{b}	79.8 ± 0.53^{b}	79.7 ± 0.38^{b}	79.5 ± 0.15^{b}	79.2 ± 0.16^{b}

Values are presented as mean$\pm$SD (n = 4 for each group). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 4. Effect of bee pollen concentration on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee Pollen (%w/w)	Moisture Content (%)
0	40.29 ± 0.11^{a}
0.075	44.20 ± 0.07^{b}
0.150	47.34 ± 0.15^{c}
0.225	51.35 ± 0.16^{d}
0.300	54.23 ± 0.15^{e}
0.375	59.25 ± 0.16^{f}

Values are presented as mean$\pm$SD (n = 4 for each group). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05)

Commented [A19]: The moisture content is quite higher than normal bread (38-40%). You may add the explanation /discussion about this

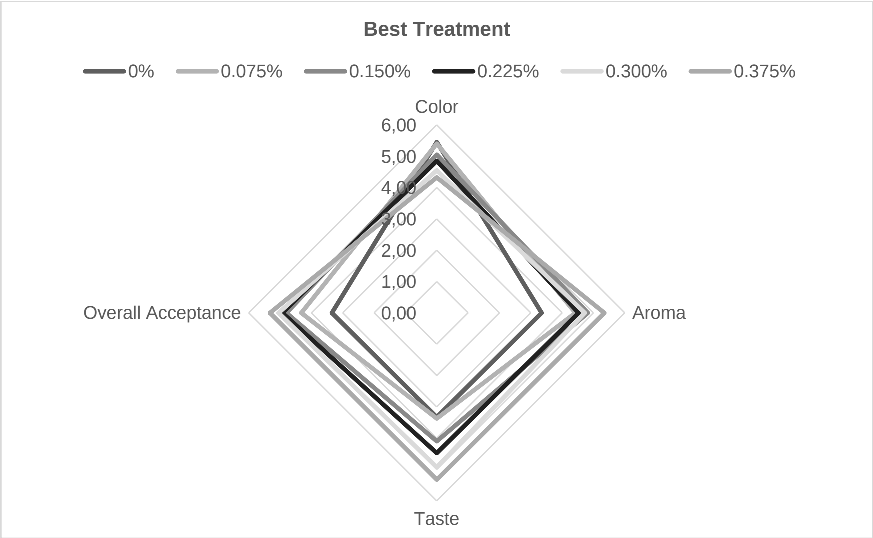

Figure 1. Spider web test result

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Evaluation Criteria	Grade				
	A (Excellent)	B	C	D	E (Worst)
1. Appropriateness of Contents		✓			
2. Originality of Topic	✓				
3. Manuscript Format	✓				
4. Research Methodology		✓			
5. Data Analysis	✓				
6. Relevance to the Journal	✓				

(REVIEWER'S SECTION) REVIEWER'S COMMENTS/SUGGESTIONS		(AUTHOR'S SECTION) AUTHOR'S ACTION/RESPONSE *NOTE FOR AUTHOR: Please state your response to the reviewer's comments/suggestion below
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2.	**Abstract** *Background, Aim, Methodology and Conclusion* Still not shown which part was improved OK	
3.	**Keywords** *Min. 3 and Max. 6* OK	
4.	**Introduction** *Concise with sufficient background* OK	
5.	**Research design/Methodology** *Clearly described and reproducible* • Material: is it necessary to write the commercial brand? (line 61-53) • Not consistent in writing the symbol of the treatments (line 78-79)	
6.	**Data Analysis** *Results well presented and discussed* • Not consistent used of "p ≤ 0,05". Some part still using α (line 4, 125, 272, 278, 285) • Not consistent in writing the symbol of the treatments, some part no symbols used (line 78-79, 266, 270, 276, 283) • The word "for each group" in line 271, 277, 284 no need to be written. • As the focus of the manuscript is improving the sensory characteristics, then the moisture content and color	

	which used as supporting parameter, should support the sensory properties discussion. Why water-soluble volatile compounds can be reduced as the amount of bee pollen increased (line 157-158)? • According to the sentence: "Textural properties was not included in the sensorial evaluation since this research was more focused on improving the taste and aroma of the bread incorporated with *Monascus*-fermented durian seeds and rice bran (line 168-170)", the texture was evaluated in this study. It is not connected to the title that want to improve the sensory properties. Sensory properties should be included texture evaluation, not only aroma and taste. • Why there is a suggestion to add hydrocolloid to improve the texture properties as there is no study about texture in this manuscript? (line 173-174)	
7.	**Conclusion** *A clear summary of the study* • The conclusion should be (line 179) related to the objective of this study (line 55-57). • What kind of food waste in this study according to line 182-186? Do it can give similar result as we use any type of food waster derived ingredients (i.e apple pomace)?	
8.	**References** *References should follow the journal's format* What is the difference between reference to reference of Koeswanto (line 220-222) and Trisnawati et al (line 260-262) as it has same title in difference language.	
9.	**English Proficiency** OK	
10.	**Additional comments/suggestions by the reviewer about the article**	

	As Figure 1 is shown not in color, please make the gradation grayscale color which is easy to understand.	

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2. Originality of Topic			x		
3. Manuscript Format			x		
4. Research Methodology				x	
5. Data Analysis				x	
6. Relevance to the Journal			X		

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2.	**Abstract** *Background, Aim, Methodology and Conclusion* Abstract is fine	
3.	**Keywords** *Min. 3 and Max. 6* The keywords is fine	
4.	**Introduction** *Concise with sufficient background* Please make it concise and focus on the topic of study. add more information on fermented durian seeds' characteristics, the problem and efforts to solve the problem	
5.	**Research design/Methodology** *Clearly described and reproducible* Bread methods are not clearly mentioned. No durian seed flour is written in the formulation. the number of parameters analysis done is quite small, and the analysis is too simple	
6.	**Data Analysis** *Results well presented and discussed* The discussion is only focused on the effect of bee pollen (while the amount in the formulation is quite small 0-3%), no discussion of the effects of other compounds and the possible interaction among compounds	
7.	**Conclusion**	

	A clear summary of the study Conclusion is fine	
8.	**References** *References should follow the journal's format* Please use more recent and related references	
9.	**English Proficiency** Required to improve the grammar	
10.	**Additional comments/suggestions by the reviewer about the article** Discuss in a deeper. Accommodate the effect of other compounds and interaction among them. If possible add some more analysis parameters to support discussion	

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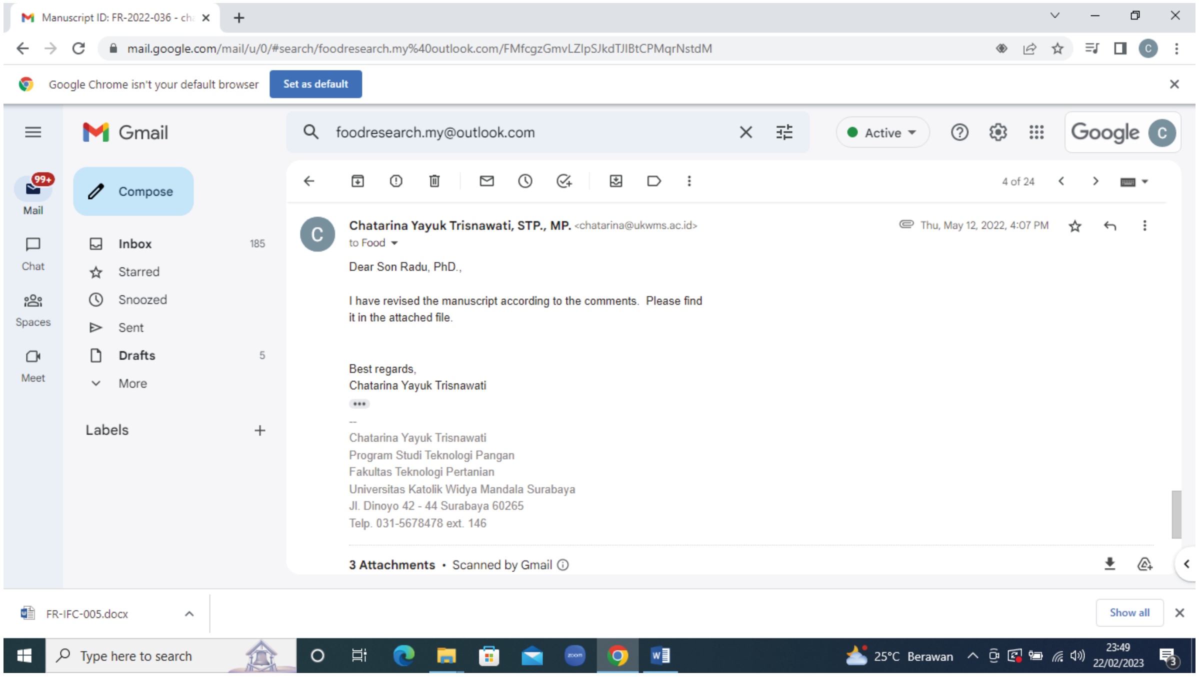

Chatarina Yayuk Trisnawati, STP., MP. <chatarina@ukwms.ac.id>
to Food

Thu, May 12, 2022, 4:07 PM

Dear Son Radu, PhD.,

I have revised the manuscript according to the comments. Please find it in the attached file.

Best regards,
Chatarina Yayuk Trisnawati

--
Chatarina Yayuk Trisnawati
Program Studi Teknologi Pangan
Fakultas Teknologi Pertanian
Universitas Katolik Widya Mandala Surabaya
Jl. Dinoyo 42 - 44 Surabaya 60265
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5. Data Analysis				x	
6. Relevance to the Journal			X		

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1.	**Title** *It should reflect the article* The title has not reflected the content of the article. Please accomodate the treatment	I've changed the title from "Improving the sensory properties of bread incorporated with Monascus-fermented durian seeds and rice bran" to "Effect of Bee Pollen on Characteristic of Bread Incorporated with *Monasus*-Fermented Durian Seeds and Rice Bran"
2.	**Abstract** *Background, Aim, Methodology and Conclusion* Abstract is fine	
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4.	**Introduction** *Concise with sufficient background* Please make it concise and focus on the topic of study. add more information on fermented durian seeds' characteristics, the problem and efforts to solve the problem	I've stated the bioactive compounds in MFDS and rice bran. I've also put the explanation regarding the problem created by incorporating MFDS and rice bran in bread and how bee pollen usage could help in solving the problem.
5.	**Research design/Methodology** *Clearly described and reproducible* Bread methods are not clearly mentioned. No durian seed flour is written in the formulation. the number of parameters analysis done is quite small, and the analysis is too simple	I've included the MFDS flour in the formulation table and added another parameter
6.	**Data Analysis** *Results well presented and discussed* The discussion is only focused on the effect of bee pollen (while the amount in the formulation is quite small 0-3%), no discussion of the effects of other compounds	I've put the effect of incorporating not only bee pollen but also rice bran and MFDS on the physicochemical properties of the bread.

	and the possible interaction among compounds	
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10.	**Additional comments/suggestions by the reviewer about the article** Discuss in a deeper. Accommodate the effect of other compounds and interaction among them. If possible add some more analysis parameters to support discussion	I've put more analyzed parameters in the paper and written the discussion for each parameter.

Overall Evaluation

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5. Data Analysis				x	
6. Relevance to the Journal			X		

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1.	**Title** *It should reflect the article* It is not reflected the article; the word "improving" is not reflect the whole article. If improving, then in the manuscript should be stated the base condition and explained what has been improved through this study.	I've changed the title from "Improving the sensory properties of bread incorporated with Monascus-fermented durian seeds and rice bran" to "Effect of Bee Pollen on Characteristic of Bread Incorporated with *Monasus-*Fermented Durian Seeds and Rice Bran"
2.	**Abstract** *Background, Aim, Methodology and Conclusion* Still not shown which part was improved OK	
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5.	**Research design/Methodology** *Clearly described and reproducible* • Material: is it necessary to write the commercial brand? (line 61-53) • Not consistent in writing the symbol of the treatments (line 78-79)	I've included the MFDS flour in the formulation table and added another parameter
6.	**Data Analysis** *Results well presented and discussed* • Not consistent used of "p>0,05". Some part still using ≤ (line 4, 125, 272, 278, 285)	I've added other parameter which were specific volume and texture analysis so there would be major change in my data analysis and discussion part.

	• Not consistent in writing the symbol of the treatments, some part no symbols used (line 78-79, 266, 270, 276, 283) • The word "for each group" in line 271, 277, 284 no need to be written. • As the focus of the manuscript is improving the sensory characteristics, then the moisture content and color which used as supporting parameter, should support the sensory properties discussion. Why water-soluble volatile compounds can be reduced as the amount of bee pollen increased (line 157- 158)? • According to the sentence: "Textural properties was not included in the sensorial evaluation since this research was more focused on improving the taste and aroma of the bread incorporated with *Monascus*-fermented durian seeds and rice bran (line 168-170)", the texture was evaluated in this study. It is not connected to the title that want to improve the sensory properties. Sensory properties should be included texture evaluation, not only aroma and taste. • Why there is a suggestion to add hydrocolloid to improve the texture properties as there is no study about texture in this manuscript? (line 173-174)	
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8.	**References**	

	References should follow the journal's format What is the difference between reference to reference of Koeswanto (line 220-222) and Trisnawati et al (line 260-262) as it has same title in difference language.	I've decided to delete Koeswanto from reference list since I did not necessarily use it for making the paper
9.	**English Proficiency** OK	
10.	As Figure 1 is shown not in color, please make the gradation grayscale color which is easy to understand.	I've put more analyzed parameters in the paper and written the discussion for each parameter.

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Effect of bee pollen on the characteristic of bread incorporated with *Monasus*-fermented durian seeds and rice bran

Goberto, M.A., *Trisnawati, C.Y., Nugerahani, I., Srianta, I. and Marsono, Y.

Department of Food Technology, Faculty of Agricultural Technology, Widya Mandala Surabaya Catholic University, Surabaya, East Java, Indonesia

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis

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of Variance with α = 5%. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB (p>0.05). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance (p>0.05). The best treatment was obtained by using 0.375% bee pollen.

Keywords: Bread, *Monascus*-fermented durian seeds, rice bran, bee pollen

1. Introduction

Commented [A2]: Please be focused on the topic, some of the paragraphs are too far from what the study focused on

Development of functional bakery products had been widely studied among food scientists as an approach for consumer's demand of baked products with extra health benefits. Trisnawati *et al.* (2019) had studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and non-dietary fiber which can regulate blood sugar level (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production. (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contains secondary metabolite such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from RB and MFDS are by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and ketons (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity flavor (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that used for producing MFDS flour were Petruk durian seeds, pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 minute and followed by adding water. Mixing process then continued for 10 minutes. Margarine and table salt then added and mixing process continued for 5 minutes. The dough was fermented at 26ºC for 30 minutes. After the dough was shaped into a loaf the dough was proofed at 26ºC for 90 minutes. The bread was baked with oven at 180ºC for 30 minutes then cooled at 26ºC for 60 minutes. Table 1 shows the composition of bread incorporated with MFDS and RB with different level of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90ºC for 10 minutes [durian seeds: 5%

Commented [A3]: You may add the formulation (the portion of each ingredient for reproducible reason)

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Commented [A5]: Please add the method you use for making bread (reference), since the mixing process you use is bit longer than regular bread dough

$Ca(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution and then washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through first drying process at 45°C for 40 minutes. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 minutes and cooled down at 26°C for 30 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 hours, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

Commented [A6]: Concentration?

Commented [A7]: Aerobic or anaerobic?

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2.3 Moisture content analysis

Moisture was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

Commented [A9]: Year?

$$\text{Moisture content (\%)}= \frac{\text{sample weight (g)}}{\text{sample weight (g)-weight loss (g)}}\times 100\%$$

2.4 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). This steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1\text{ (ml)-}V_2\text{ (ml)}}{\text{W (g)}}$$

2.5 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková et al., 2012).

2.6 Color analysis

Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm x 5 cm x 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

Commented [A10]: Most of the discussions are only focused on the bee pollen (with a concentration only 0-3%), please accommodate many other compounds and their interaction that may contribute to the product quality

3.1 Moisture Content

According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid. (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease bread's springiness.

The results in Table 5. showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since it is brown in color and Maillard reaction which occurred during baking process. Starowicz and Zieliński (2019) reported that Free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L*value of food Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value of b* was also influenced by the yellow pigment β-carotene in bee pollen.

3.3 Sensory evaluation

The preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web test showed that 0.375% BP was the best concentration for bread incorporated wih MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which derived from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen; B_1 : 0.075% bee pollen; B_2 : 0.150% bee pollen; B_3 : 0.225% bee pollen; B_4 : 0.300% bee pollen; B_5 : 0.375% bee pollen

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Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Moisture Content (%)
B_0	40.29±0.11[a]
B_1	44.20±0.07[b]
B_2	47.34±0.15[c]
B_3	51.35±0.16[d]
B_4	54.23±0.15[e]
B_5	59.25±0.16[f]

Commented [A13]: The moisture content is quite higher than normal bread (38-40%). You may add the explanation /discussion about this

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85±0.03[c]
B_1	3.97±0.01[d]
B_2	4.07±0.06[e]
B_3	3.81±0.02[c]
B_4	3.34±0.06[b]
B_5	3.23±0.05[a]

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 $\pm 7.920^c$	769.631 $\pm 10.910^b$	665.708 $\pm 5.590^a$	833.009 $\pm 10.450^d$	1059.045 $\pm 17.130^e$	1254.505 $\pm 15.140^f$
Cohesiveness	0.643 $\pm 0.010^c$	0.662 $\pm 0.010^d$	0.677 $\pm 0.010^e$	0.646 $\pm 0.010^c$	0.620 $\pm 0.010^b$	0.596 $\pm 0.010^a$
Springiness	0.790 $\pm 0.011^c$	0.819 $\pm 0.003^d$	0.833 $\pm 0.005^e$	0.802 $\pm 0.001^d$	0.768 $\pm 0.002^b$	0.758 $\pm 0.003^a$

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2 ± 0.25^d	67.6 ± 0.39^c	66.8 ± 0.99^b	66.5 ± 0.20^b	65.7 ± 0.55^a	65.3 ± 0.17^a
Redness (a*)	3.2 ± 0.13^a	3.2 ± 0.10^a	3.3 ± 0.18^a	3.4 ± 0.13^b	3.6 ± 0.10^c	3.8 ± 0.03^d
Yellowness (b*)	15.9 ± 0.13^a	17.4 ± 0.28^b	18.2 ± 0.22^c	18.6 ± 0.45^c	19.5 ± 0.45^d	20.1 ± 0.32^e
Chroma (C)	16.2 ± 0.15^a	17.7 ± 0.28^b	18.5 ± 0.22^c	18.9 ± 0.45^c	19.8 ± 0.46^d	20.5 ± 0.33^e
Hue (°H)	78.7 ± 0.43^a	79.6 ± 0.48^b	79.8 ± 0.53^b	79.7 ± 0.38^b	79.5 ± 0.15^b	79.2 ± 0.16^b

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45 ± 1.20^{d}	5.40 ± 0.93^{d}	5.05 ± 1.20^{cd}	4.85 ± 0.98^{bc}	4.55 ± 1.15^{ab}	4.33 ± 1.54^{a}
Aroma	3.35 ± 0.92^{a}	4.43 ± 1.36^{b}	4.83 ± 1.01^{b}	4.53 ± 1.22^{b}	4.73 ± 1.26^{b}	5.35 ± 0.95^{c}
Taste	3.33 ± 0.92^{a}	3.38 ± 0.90^{a}	4.10 ± 1.01^{b}	4.48 ± 1.26^{b}	4.93 ± 1.23^{c}	5.33 ± 0.76^{d}
Overall acceptance	3.35 ± 0.77^{a}	4.33 ± 1.05^{b}	4.80 ± 1.18^{c}	4.93 ± 1.21^{cd}	5.05 ± 1.20^{cd}	5.33 ± 0.97^{d}

Values are presented as mean$\pm$SD (n = 50). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

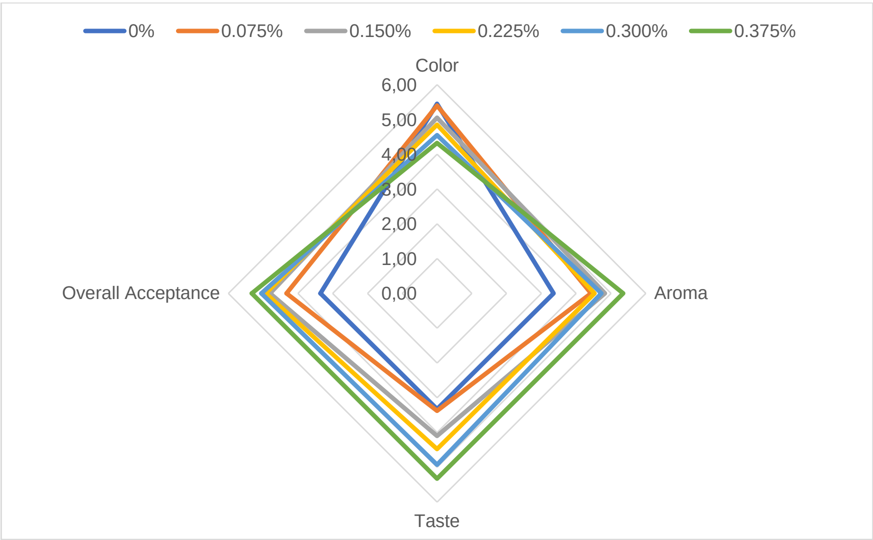

Figure 1. Spider web test result

4.

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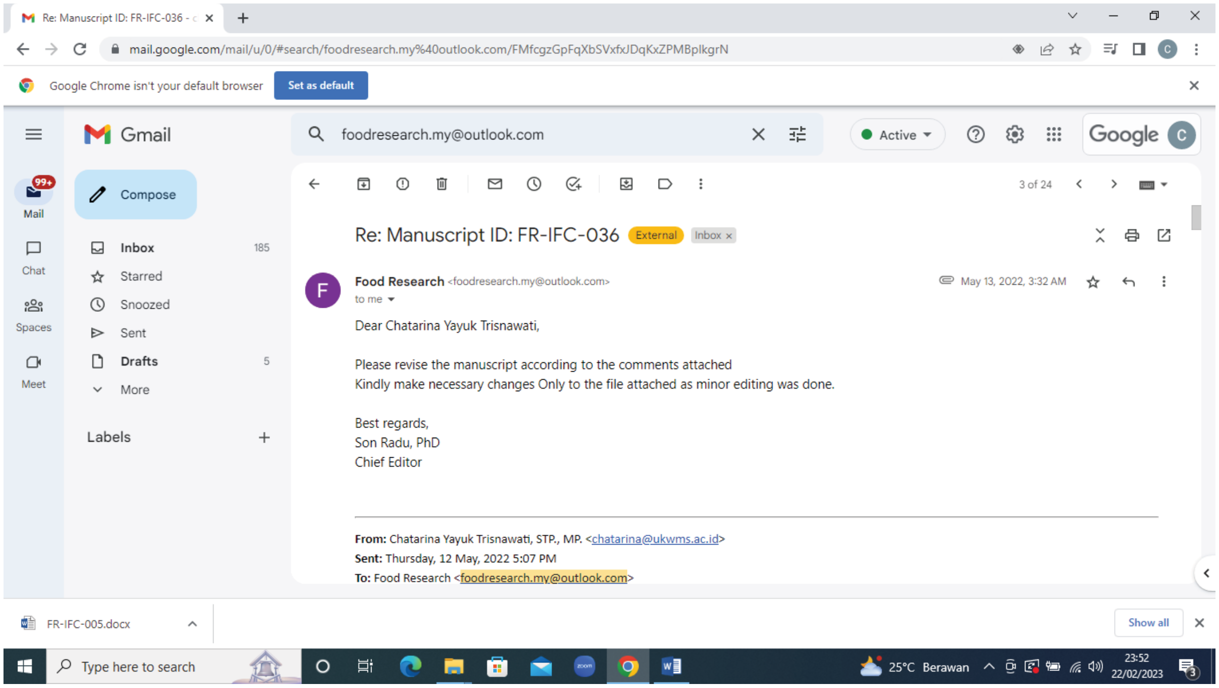

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Effect of bee pollen on the characteristic of bread incorporated with *Monasus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis

of Variance with α = 5%. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB (p>0.05). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance (p>0.05). The best treatment was obtained by using 0.375% bee pollen.

Keywords: Bread, *Monascus*-fermented durian seeds, rice bran, bee pollen

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and non-dietary fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production. (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and ketons (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity

flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 minute and followed by adding water. The mixing process then continued for 10 minutes. Margarine and table salt was then added and the mixing process continued for 5 minutes. The dough was fermented at 26^{o}C for 30 minutes. After the dough was shaped into a loaf the dough was proofed at 26^{o}C for 90 minutes. The bread was baked with the oven at 180^{o}C for 30 minutes then cooled at 26^{o}C for 60 minutes. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90°C for 10 minutes [durian seeds: 5% $Ca(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution and then washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through first drying process at 45°C for 40 minutes. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 minutes and cooled down at 26°C for 30 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 hours, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

2.3 Moisture content analysis

Moisture was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)} - \text{weight loss (g)}} \times 100\%$$

2.4 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). This steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1 \text{ (ml)} - V_2 \text{ (ml)}}{\text{W (g)}}$$

2.5 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková et al., 2012).

2.6 Color analysis

Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm x 5 cm x 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture Content

According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid. (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease bread's springiness.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since it is brown in color and Maillard reaction which occurred during baking process. Starowicz and Zieliński (2019) reported that Free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L*value of food Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value of b* was also influenced by the yellow pigment β-carotene in bee pollen.

3.3 Sensory evaluation

The preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web test showed that 0.375% BP was the best concentration for bread incorporated wih MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which derived from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen; B_1 : 0.075% bee pollen; B_2 : 0.150% bee pollen; B_3 : 0.225% bee pollen; B_4 : 0.300% bee pollen; B_5 : 0.375% bee pollen

Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Moisture Content (%)
B_0	40.29±0.11[a]
B_1	44.20±0.07[b]
B_2	47.34±0.15[c]
B_3	51.35±0.16[d]
B_4	54.23±0.15[e]
B_5	59.25±0.16[f]

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran

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Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85±0.03[c]
B_1	3.97±0.01[d]
B_2	4.07±0.06[e]
B_3	3.81±0.02[c]
B_4	3.34±0.06[b]
B_5	3.23±0.05[a]

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 $\pm$7.920^{c}	769.631 $\pm$10.910^{b}	665.708 $\pm$5.590^{a}	833.009 $\pm$10.450^{d}	1059.045 $\pm$17.130^{e}	1254.505 $\pm$15.140^{f}
Cohesiveness	0.643 $\pm$0.010^{c}	0.662 $\pm$0.010^{d}	0.677 $\pm$0.010^{e}	0.646 $\pm$0.010^{c}	0.620 $\pm$0.010^{b}	0.596 $\pm$0.010^{a}
Springiness	0.790 $\pm$0.011^{c}	0.819 $\pm$0.003^{d}	0.833 $\pm$0.005^{e}	0.802 $\pm$0.001^{d}	0.768 $\pm$0.002^{b}	0.758 $\pm$0.003^{a}

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2$\pm$0.25^{d}	67.6$\pm$0.39^{c}	66.8$\pm$0.99^{b}	66.5$\pm$0.20^{b}	65.7$\pm$0.55^{a}	65.3$\pm$0.17^{a}
Redness (a*)	3.2$\pm$0.13^{a}	3.2$\pm$0.10^{a}	3.3$\pm$0.18^{a}	3.4$\pm$0.13^{b}	3.6$\pm$0.10^{c}	3.8$\pm$0.03^{d}
Yellowness (b*)	15.9$\pm$0.13^{a}	17.4$\pm$0.28^{b}	18.2$\pm$0.22^{c}	18.6$\pm$0.45^{c}	19.5$\pm$0.45^{d}	20.1$\pm$0.32^{e}
Chroma (C)	16.2$\pm$0.15^{a}	17.7$\pm$0.28^{b}	18.5$\pm$0.22^{c}	18.9$\pm$0.45^{c}	19.8$\pm$0.46^{d}	20.5$\pm$0.33^{e}
Hue (^{o}H)	78.7$\pm$0.43^{a}	79.6$\pm$0.48^{b}	79.8$\pm$0.53^{b}	79.7$\pm$0.38^{b}	79.5$\pm$0.15^{b}	79.2$\pm$0.16^{b}

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

272 Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45 ± 1.20^{d}	5.40 ± 0.93^{d}	5.05 ± 1.20^{cd}	4.85 ± 0.98^{bc}	4.55 ± 1.15^{ab}	4.33 ± 1.54^{a}
Aroma	3.35 ± 0.92^{a}	4.43 ± 1.36^{b}	4.83 ± 1.01^{b}	4.53 ± 1.22^{b}	4.73 ± 1.26^{b}	5.35 ± 0.95^{c}
Taste	3.33 ± 0.92^{a}	3.38 ± 0.90^{a}	4.10 ± 1.01^{b}	4.48 ± 1.26^{b}	4.93 ± 1.23^{c}	5.33 ± 0.76^{d}
Overall acceptance	3.35 ± 0.77^{a}	4.33 ± 1.05^{b}	4.80 ± 1.18^{c}	4.93 ± 1.21^{cd}	5.05 ± 1.20^{cd}	5.33 ± 0.97^{d}

274 Values are presented as mean$\pm$SD (n = 50). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

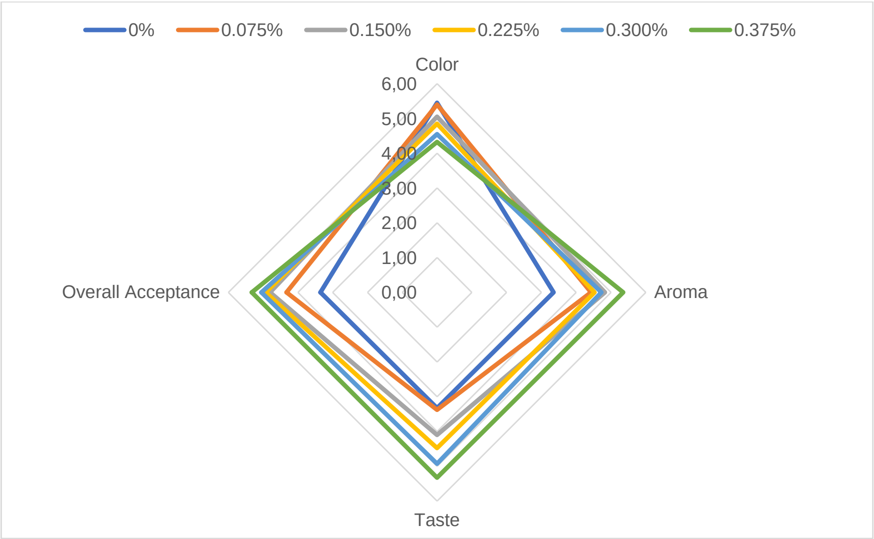

Figure 1. Spider web test result

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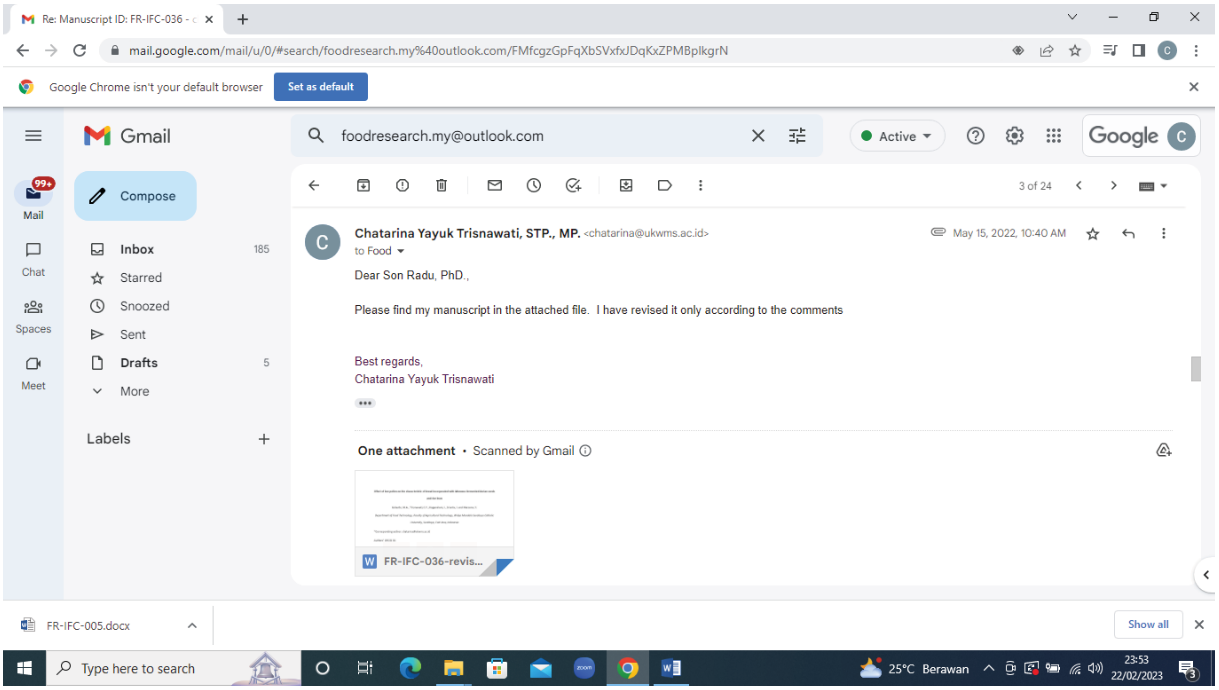
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Effect of bee pollen on the characteristic of bread incorporated with *Monasus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis

of Variance with α = 5%. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB (p>0.05). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance (p>0.05). The best treatment was obtained by using 0.375% bee pollen.

Keywords: Bread, *Monascus*-fermented durian seeds, rice bran, bee pollen

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production. (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and keton (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity

flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 minute and followed by adding water. The mixing process then continued for 10 minutes. Margarine and table salt was then added and the mixing process continued for 5 minutes. The dough was fermented at 26^{o}C for 30 minutes. After the dough was shaped into a loaf the dough was proofed at 26^{o}C for 90 minutes. The bread was baked with the oven at 180^{o}C for 30 minutes then cooled at 26^{o}C for 60 minutes. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90^{o}C for 10 minutes [durian seeds: 5%

$Ca(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution and then washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through first drying process at 45°C for 40 minutes. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 minutes and cooled down at 26°C for 30 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30$\pm$1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 hours, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

2.3 Moisture content analysis

Moisture content determination was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)-weight loss (g)}} \times 100\%$$

2.4 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). These steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1 \text{ (ml)} - V_2 \text{ (ml)}}{W \text{ (g)}}$$

2.5 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková et al., 2012).

2.6 Color analysis

Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm x 5 cm x 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with $\alpha = 5\%$ and followed by Duncan's Multiple Range Test (DMRT) with $\alpha = 5\%$ using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture Content

According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid. (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease bread's springiness.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since it is brown in color and Maillard reaction which occurred during baking process.

Starowicz and Zieliński (2019) reported that free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L* value of food. Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value of b* was also influenced by the yellow pigment β-carotene in bee pollen.

3.3 Sensory evaluation

Table 6. showed that the preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web in Figure 1. test showed that 0.375% BP was the best concentration for bread incorporated with MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which derived from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen; B_1 : 0.075% bee pollen; B_2 : 0.150% bee pollen; B_3 : 0.225% bee pollen; B_4 : 0.300% bee pollen; B_5 : 0.375% bee pollen

Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Moisture Content (%)
B_0	40.29 ± 0.11^a
B_1	44.20 ± 0.07^b
B_2	47.34 ± 0.15^c
B_3	51.35 ± 0.16^d
B_4	54.23 ± 0.15^e
B_5	59.25 ± 0.16^f

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran

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Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85 ± 0.03^c
B_1	3.97 ± 0.01^d
B_2	4.07 ± 0.06^e
B_3	3.81 ± 0.02^c
B_4	3.34 ± 0.06^b
B_5	3.23 ± 0.05^a

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 $\pm$7.920^c	769.631 $\pm$10.910^b	665.708 $\pm$5.590^a	833.009 $\pm$10.450^d	1059.045 $\pm$17.130^e	1254.505 $\pm$15.140^f
Cohesiveness	0.643 $\pm$0.010^c	0.662 $\pm$0.010^d	0.677 $\pm$0.010^e	0.646 $\pm$0.010^c	0.620 $\pm$0.010^b	0.596 $\pm$0.010^a
Springiness	0.790 $\pm$0.011^c	0.819 $\pm$0.003^d	0.833 $\pm$0.005^e	0.802 $\pm$0.001^d	0.768 $\pm$0.002^b	0.758 $\pm$0.003^a

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2$\pm$0.25^d	67.6$\pm$0.39^c	66.8$\pm$0.99^b	66.5$\pm$0.20^b	65.7$\pm$0.55^a	65.3$\pm$0.17^a
Redness (a*)	3.2$\pm$0.13^a	3.2$\pm$0.10^a	3.3$\pm$0.18^a	3.4$\pm$0.13^b	3.6$\pm$0.10^c	3.8$\pm$0.03^d
Yellowness (b*)	15.9$\pm$0.13^a	17.4$\pm$0.28^b	18.2$\pm$0.22^c	18.6$\pm$0.45^c	19.5$\pm$0.45^d	20.1$\pm$0.32^e
Chroma (C)	16.2$\pm$0.15^a	17.7$\pm$0.28^b	18.5$\pm$0.22^c	18.9$\pm$0.45^c	19.8$\pm$0.46^d	20.5$\pm$0.33^e
Hue (^{o}H)	78.7$\pm$0.43^a	79.6$\pm$0.48^b	79.8$\pm$0.53^b	79.7$\pm$0.38^b	79.5$\pm$0.15^b	79.2$\pm$0.16^b

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45 ± 1.20^{d}	5.40 ± 0.93^{d}	5.05 ± 1.20^{cd}	4.85 ± 0.98^{bc}	4.55 ± 1.15^{ab}	4.33 ± 1.54^{a}
Aroma	3.35 ± 0.92^{a}	4.43 ± 1.36^{b}	4.83 ± 1.01^{b}	4.53 ± 1.22^{b}	4.73 ± 1.26^{b}	5.35 ± 0.95^{c}
Taste	3.33 ± 0.92^{a}	3.38 ± 0.90^{a}	4.10 ± 1.01^{b}	4.48 ± 1.26^{b}	4.93 ± 1.23^{c}	5.33 ± 0.76^{d}
Overall acceptance	3.35 ± 0.77^{a}	4.33 ± 1.05^{b}	4.80 ± 1.18^{c}	4.93 ± 1.21^{cd}	5.05 ± 1.20^{cd}	5.33 ± 0.97^{d}

Values are presented as mean$\pm$SD (n = 50). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

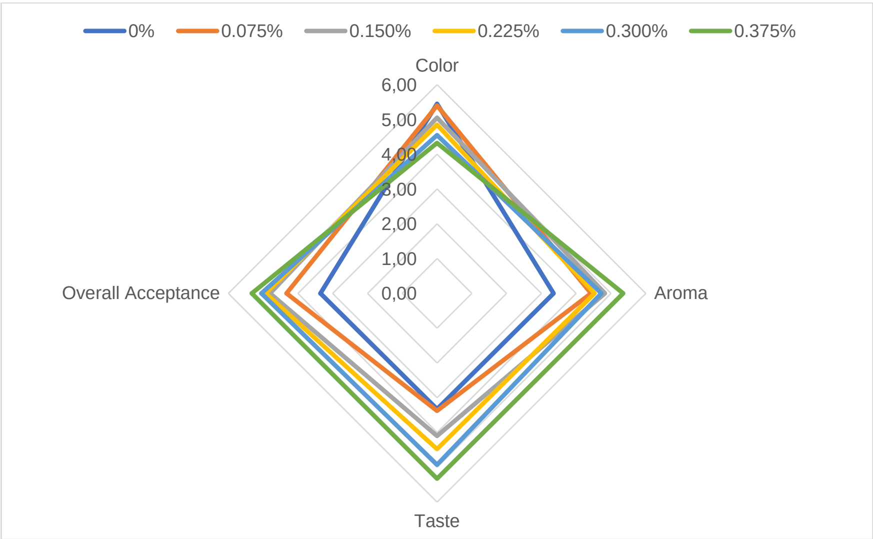

Figure 1. Spider web test result

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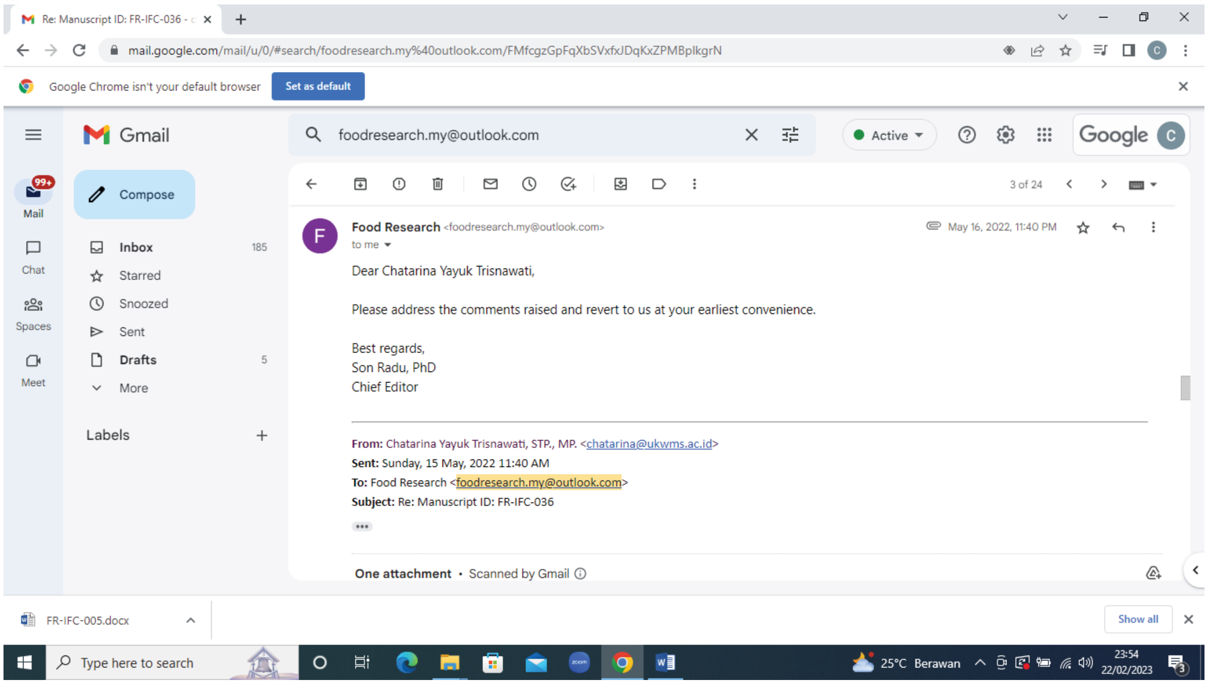
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Effect of bee pollen on the characteristic of bread incorporated with *Monasus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis

of Variance with α = 5%. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB ($p>0.05$). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance ($p>0.05$). The best treatment was obtained by using 0.375% bee pollen.

Keywords: Bread, *Monascus*-fermented durian seeds, rice bran, bee pollen

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production. (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

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Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and keton (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity

flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 minute and followed by adding water. The mixing process then continued for 10 minutes. Margarine and table salt was then added and the mixing process continued for 5 minutes. The dough was fermented at 26^{o}C for 30 minutes. After the dough was shaped into a loaf the dough was proofed at 26^{o}C for 90 minutes. The bread was baked with the oven at 180^{o}C for 30 minutes then cooled at 26^{o}C for 60 minutes. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

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2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90^{o}C for 10 minutes [durian seeds: 5%

$Ca(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution and then washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through first drying process at 45°C for 40 minutes. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 minutes and cooled down at 26°C for 30 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 hours, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

2.3 Moisture content analysis

Moisture content determination was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)-weight loss (g)}} \times 100\%$$

2.4 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). These steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1\text{ (ml)-}V_2\text{ (ml)}}{\text{W (g)}}$$

2.5 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková *et al*., 2012).

2.6 Color analysis

Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm x 5 cm x 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture Content

According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid. (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease bread's springiness.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since it is brown in color and Maillard reaction which occurred during baking process.

Starowicz and Zieliński (2019) reported that free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L* value of food. Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value of b* was also influenced by the yellow pigment β-carotene in bee pollen.

3.3 Sensory evaluation

Table 6. showed that the preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web in Figure 1. test showed that 0.375% BP was the best concentration for bread incorporated with MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which derived from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen; B_1 : 0.075% bee pollen; B_2 : 0.150% bee pollen; B_3 : 0.225% bee pollen; B_4 : 0.300% bee pollen; B_5 : 0.375% bee pollen

Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Moisture Content (%)
B_0	40.29 ± 0.11^{a}
B_1	44.20 ± 0.07^{b}
B_2	47.34 ± 0.15^{c}
B_3	51.35 ± 0.16^{d}
B_4	54.23 ± 0.15^{e}
B_5	59.25 ± 0.16^{f}

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85 ± 0.03^{c}
B_1	3.97 ± 0.01^{d}
B_2	4.07 ± 0.06^{e}
B_3	3.81 ± 0.02^{c}
B_4	3.34 ± 0.06^{b}
B_5	3.23 ± 0.05^{a}

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 $\pm 7.920^c$	769.631 $\pm 10.910^b$	665.708 $\pm 5.590^a$	833.009 $\pm 10.450^d$	1059.045 $\pm 17.130^e$	1254.505 $\pm 15.140^f$
Cohesiveness	0.643 $\pm 0.010^c$	0.662 $\pm 0.010^d$	0.677 $\pm 0.010^e$	0.646 $\pm 0.010^c$	0.620 $\pm 0.010^b$	0.596 $\pm 0.010^a$
Springiness	0.790 $\pm 0.011^c$	0.819 $\pm 0.003^d$	0.833 $\pm 0.005^e$	0.802 $\pm 0.001^d$	0.768 $\pm 0.002^b$	0.758 $\pm 0.003^a$

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2 ± 0.25^d	67.6 ± 0.39^c	66.8 ± 0.99^b	66.5 ± 0.20^b	65.7 ± 0.55^a	65.3 ± 0.17^a
Redness (a*)	3.2 ± 0.13^a	3.2 ± 0.10^a	3.3 ± 0.18^a	3.4 ± 0.13^b	3.6 ± 0.10^c	3.8 ± 0.03^d
Yellowness (b*)	15.9 ± 0.13^a	17.4 ± 0.28^b	18.2 ± 0.22^c	18.6 ± 0.45^c	19.5 ± 0.45^d	20.1 ± 0.32^e
Chroma (C)	16.2 ± 0.15^a	17.7 ± 0.28^b	18.5 ± 0.22^c	18.9 ± 0.45^c	19.8 ± 0.46^d	20.5 ± 0.33^e
Hue (°H)	78.7 ± 0.43^a	79.6 ± 0.48^b	79.8 ± 0.53^b	79.7 ± 0.38^b	79.5 ± 0.15^b	79.2 ± 0.16^b

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45 ± 1.20^{d}	5.40 ± 0.93^{d}	5.05 ± 1.20^{cd}	4.85 ± 0.98^{bc}	4.55 ± 1.15^{ab}	4.33 ± 1.54^{a}
Aroma	3.35 ± 0.92^{a}	4.43 ± 1.36^{b}	4.83 ± 1.01^{b}	4.53 ± 1.22^{b}	4.73 ± 1.26^{b}	5.35 ± 0.95^{c}
Taste	3.33 ± 0.92^{a}	3.38 ± 0.90^{a}	4.10 ± 1.01^{b}	4.48 ± 1.26^{b}	4.93 ± 1.23^{c}	5.33 ± 0.76^{d}
Overall acceptance	3.35 ± 0.77^{a}	4.33 ± 1.05^{b}	4.80 ± 1.18^{c}	4.93 ± 1.21^{cd}	5.05 ± 1.20^{cd}	5.33 ± 0.97^{d}

Values are presented as mean±SD (n = 50). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

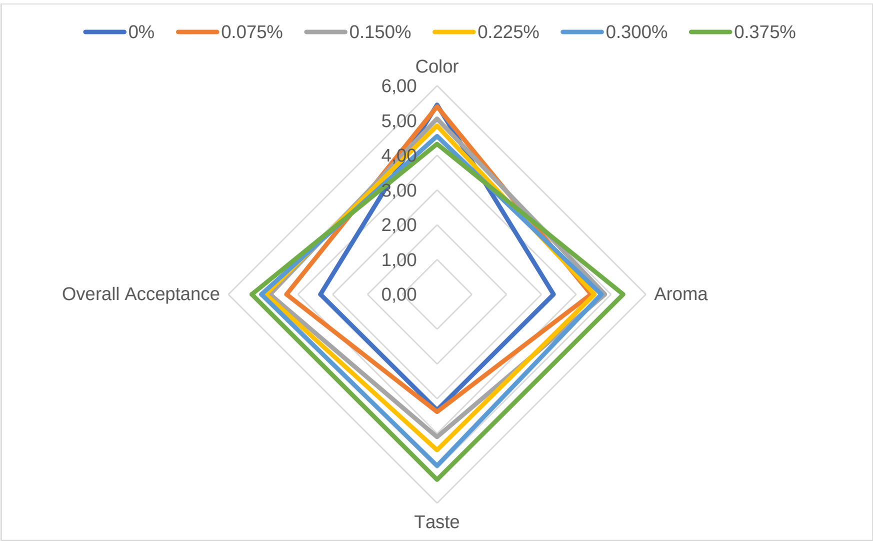

Figure 1. Spider web test result

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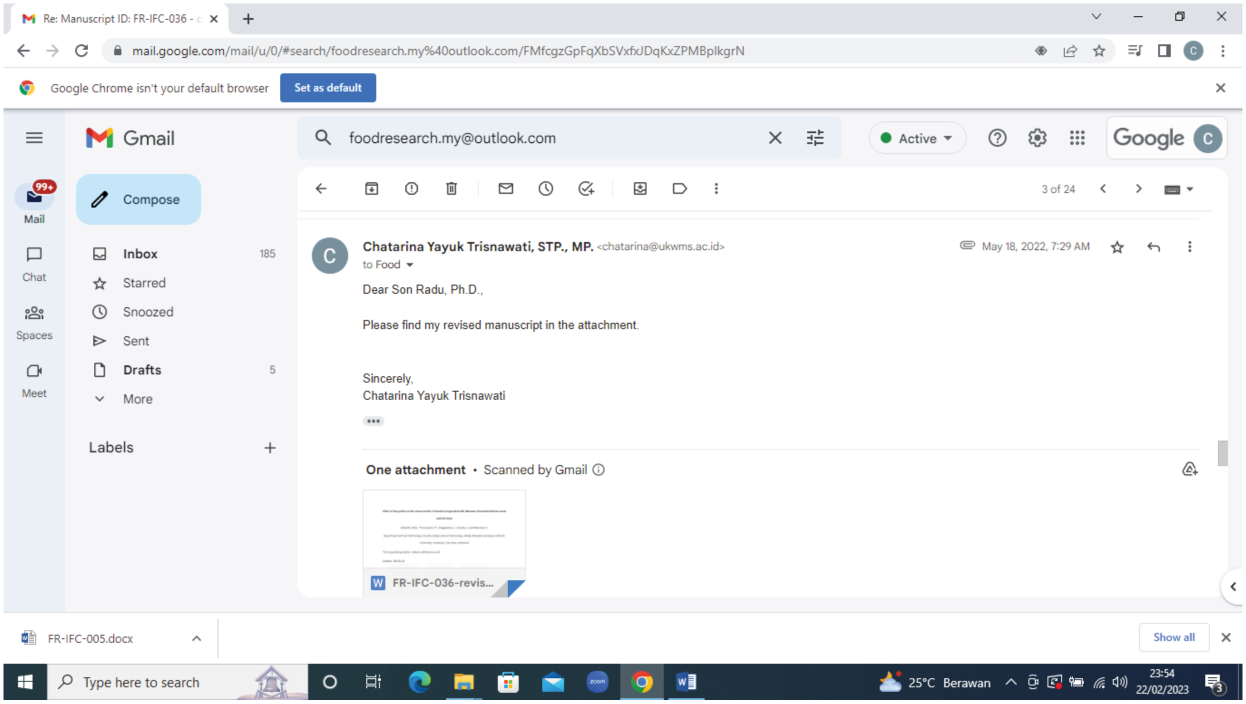

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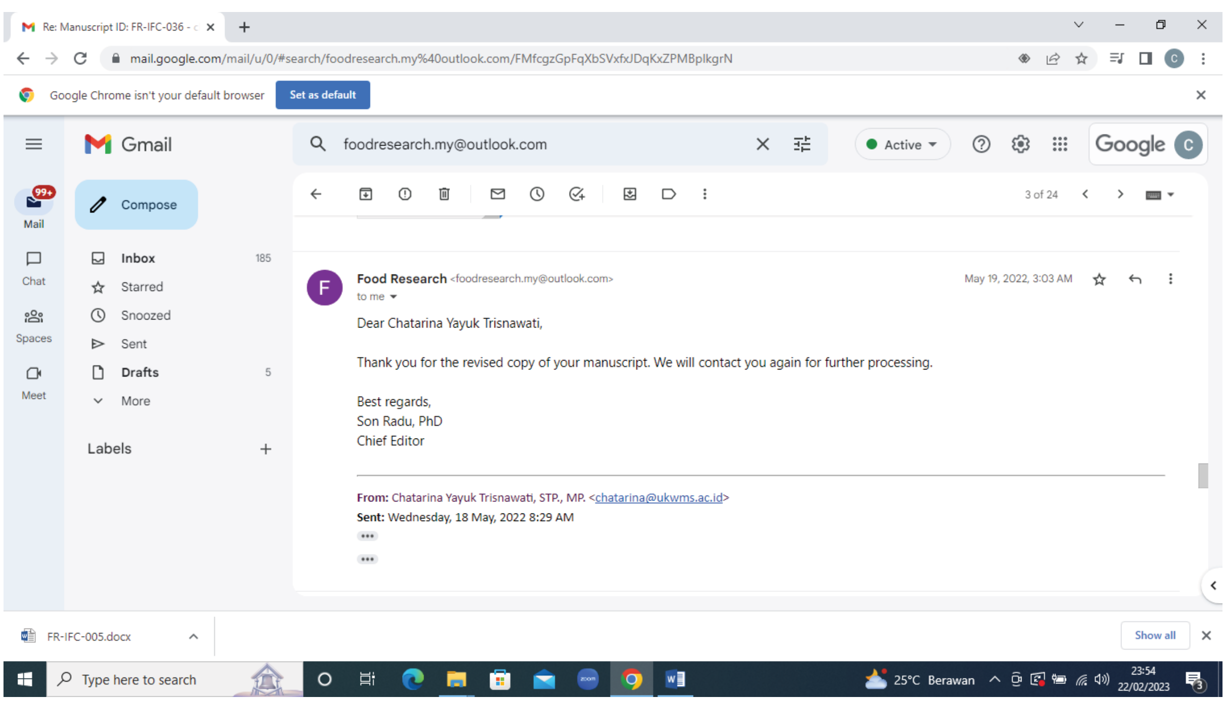

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Effect of bee pollen on the characteristic of bread incorporated with *Monasus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis

of Variance with α = 5%. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB (p>0.05). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance (p>0.05). The best treatment was obtained by using 0.375% bee pollen.

Keywords: Bread, *Monascus*-fermented durian seeds, rice bran, bee pollen

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production. (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

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Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and keton (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity

flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 min~~ute~~ and followed by adding water. The mixing process then continued for 10 min~~ute~~s. Margarine and table salt was then added and the mixing process continued for 5 min~~nute~~s. The dough was fermented at 26°C for 30 min~~ute~~s. After the dough was shaped into a loaf the dough was proofed at 26°C for 90 min~~ute~~s. The bread was baked with the oven at 180°C for 30 min~~ute~~s then cooled at 26°C for 60 min~~ute~~s. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90°C for 10 min~~ute~~s [durian seeds: 5%

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$Ca(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution and then washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through first drying process at 45°C for 40 minutes. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 minutes and cooled down at 26°C for 30 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 hours, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

2.3 Moisture content analysis

Moisture content determination was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)-weight loss (g)}} \times 100\%$$

2.4 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). These steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1\text{ (ml)}-V_2\text{ (ml)}}{\text{W (g)}}$$

2.5 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková *et al*., 2012).

2.6 Color analysis

Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (^{o}H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm x 5 cm x 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture Content

According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid. (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease bread's springiness.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since it is brown in color and Maillard reaction which occurred during baking process.

Starowicz and Zieliński (2019) reported that free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L* value of food. Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value of b* was also influenced by the yellow pigment β-carotene in bee pollen.

3.3 Sensory evaluation

Table 6. showed that the preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web in Figure 1. test showed that 0.375% BP was the best concentration for bread incorporated with MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which derived from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen; B_1 : 0.075% bee pollen; B_2 : 0.150% bee pollen; B_3 : 0.225% bee pollen; B_4 : 0.300% bee pollen; B_5 : 0.375% bee pollen

Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Moisture Content (%)
B_0	40.29±0.11[a]
B_1	44.20±0.07[b]
B_2	47.34±0.15[c]
B_3	51.35±0.16[d]
B_4	54.23±0.15[e]
B_5	59.25±0.16[f]

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85±0.03[c]
B_1	3.97±0.01[d]
B_2	4.07±0.06[e]
B_3	3.81±0.02[c]
B_4	3.34±0.06[b]
B_5	3.23±0.05[a]

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 ±7.920^c	769.631 ±10.910^b	665.708 ±5.590^a	833.009 ±10.450^d	1059.045 ±17.130^e	1254.505 ±15.140^f
Cohesiveness	0.643 ±0.010^c	0.662 ±0.010^d	0.677 ±0.010^e	0.646 ±0.010^c	0.620 ±0.010^b	0.596 ±0.010^a
Springiness	0.790 ±0.011^c	0.819 ±0.003^d	0.833 ±0.005^e	0.802 ±0.001^d	0.768 ±0.002^b	0.758 ±0.003^a

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2±0.25^d	67.6±0.39^c	66.8±0.99^b	66.5±0.20^b	65.7±0.55^a	65.3±0.17^a
Redness (a*)	3.2±0.13^a	3.2±0.10^a	3.3±0.18^a	3.4±0.13^b	3.6±0.10^c	3.8±0.03^d
Yellowness (b*)	15.9±0.13^a	17.4±0.28^b	18.2±0.22^c	18.6±0.45^c	19.5±0.45^d	20.1±0.32^e
Chroma (C)	16.2±0.15^a	17.7±0.28^b	18.5±0.22^c	18.9±0.45^c	19.8±0.46^d	20.5±0.33^e
Hue (°H)	78.7±0.43^a	79.6±0.48^b	79.8±0.53^b	79.7±0.38^b	79.5±0.15^b	79.2±0.16^b

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45 ± 1.20^{d}	5.40 ± 0.93^{d}	5.05 ± 1.20^{cd}	4.85 ± 0.98^{bc}	4.55 ± 1.15^{ab}	4.33 ± 1.54^{a}
Aroma	3.35 ± 0.92^{a}	4.43 ± 1.36^{b}	4.83 ± 1.01^{b}	4.53 ± 1.22^{b}	4.73 ± 1.26^{b}	5.35 ± 0.95^{c}
Taste	3.33 ± 0.92^{a}	3.38 ± 0.90^{a}	4.10 ± 1.01^{b}	4.48 ± 1.26^{b}	4.93 ± 1.23^{c}	5.33 ± 0.76^{d}
Overall acceptance	3.35 ± 0.77^{a}	4.33 ± 1.05^{b}	4.80 ± 1.18^{c}	4.93 ± 1.21^{cd}	5.05 ± 1.20^{cd}	5.33 ± 0.97^{d}

Values are presented as mean$\pm$SD (n = 50). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

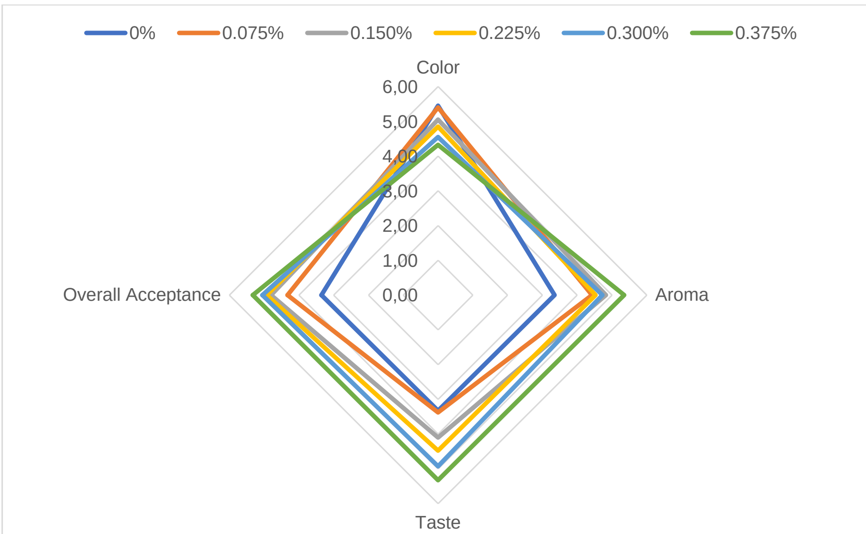

Figure 1. Spider web test result

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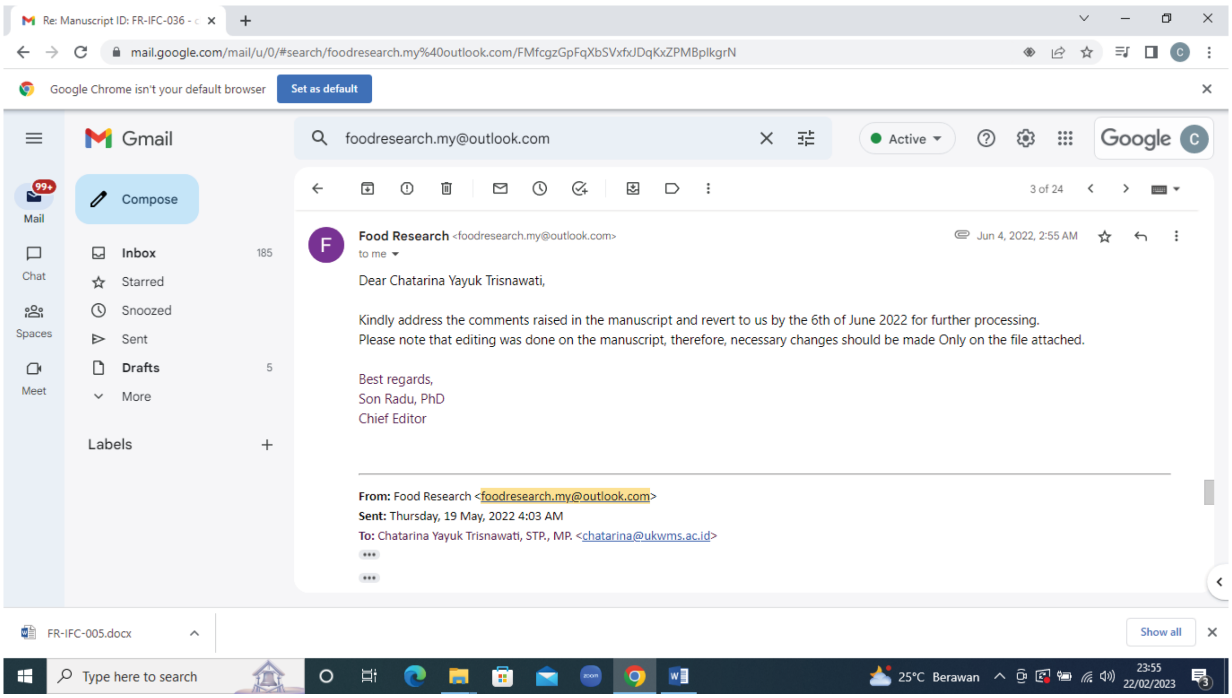

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Effect of bee pollen on the characteristic of bread incorporated with *Monasus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis

of Variance with α = 5%. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB (p>0.05). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance (p>0.05). The best treatment was obtained by using 0.375% bee pollen.

Keywords: Bread, *Monascus*-fermented durian seeds, Rice bran, Bee pollen

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production. (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and keton (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity

flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 min and followed by adding water. The mixing process then continued for 10 mins. Margarine and table salt was then added and the mixing process continued for 5 mins. The dough was fermented at 26^{o}C for 30 mins. After the dough was shaped into a loaf the dough was proofed at 26^{o}C for 90 mins. The bread was baked with the oven at 180^{o}C for 30 mins then cooled at 26^{o}C for 60 mins. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90^{o}C for 10 mins [durian seeds: 5%

$Ca(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution and then washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through first drying process at 45°C for 40 mins. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 mins and cooled down at 26°C for 30 mins. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30$\pm$1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 h, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

2.3 Moisture content analysis

Moisture content determination was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)-weight loss (g)}} \times 100\%$$

2.4 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). These steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1\text{ (ml)-}V_2\text{ (ml)}}{\text{W (g)}}$$

2.5 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková *et al*., 2012).

2.6 Color analysis

Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (^{o}H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm x 5 cm x 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture Content

According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease bread's springiness.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since it is brown in color and Maillard reaction which occurred during baking process.

Starowicz and Zieliński (2019) reported that free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L* value of food. Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value of b* was also influenced by the yellow pigment β-carotene in bee pollen.

3.3 Sensory evaluation

Table 6. showed that the preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web in Figure 1. test showed that 0.375% BP was the best concentration for bread incorporated with MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which derived from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen; B_1 : 0.075% bee pollen; B_2 : 0.150% bee pollen; B_3 : 0.225% bee pollen; B_4 : 0.300% bee pollen; B_5 : 0.375% bee pollen

Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Moisture Content (%)
B_0	40.29 ± 0.11^{a}
B_1	44.20 ± 0.07^{b}
B_2	47.34 ± 0.15^{c}
B_3	51.35 ± 0.16^{d}
B_4	54.23 ± 0.15^{e}
B_5	59.25 ± 0.16^{f}

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85 ± 0.03^{c}
B_1	3.97 ± 0.01^{d}
B_2	4.07 ± 0.06^{e}
B_3	3.81 ± 0.02^{c}
B_4	3.34 ± 0.06^{b}
B_5	3.23 ± 0.05^{a}

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 $\pm7.920^{c}$	769.631 $\pm10.910^{b}$	665.708 $\pm5.590^{a}$	833.009 $\pm10.450^{d}$	1059.045 $\pm17.130^{e}$	1254.505 $\pm15.140^{f}$
Cohesiveness	0.643 $\pm0.010^{c}$	0.662 $\pm0.010^{d}$	0.677 $\pm0.010^{e}$	0.646 $\pm0.010^{c}$	0.620 $\pm0.010^{b}$	0.596 $\pm0.010^{a}$
Springiness	0.790 $\pm0.011^{c}$	0.819 $\pm0.003^{d}$	0.833 $\pm0.005^{e}$	0.802 $\pm0.001^{d}$	0.768 $\pm0.002^{b}$	0.758 $\pm0.003^{a}$

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2 ± 0.25^{d}	67.6 ± 0.39^{c}	66.8 ± 0.99^{b}	66.5 ± 0.20^{b}	65.7 ± 0.55^{a}	65.3 ± 0.17^{a}
Redness (a*)	3.2 ± 0.13^{a}	3.2 ± 0.10^{a}	3.3 ± 0.18^{a}	3.4 ± 0.13^{b}	3.6 ± 0.10^{c}	3.8 ± 0.03^{d}
Yellowness (b*)	15.9 ± 0.13^{a}	17.4 ± 0.28^{b}	18.2 ± 0.22^{c}	18.6 ± 0.45^{c}	19.5 ± 0.45^{d}	20.1 ± 0.32^{e}
Chroma (C)	16.2 ± 0.15^{a}	17.7 ± 0.28^{b}	18.5 ± 0.22^{c}	18.9 ± 0.45^{c}	19.8 ± 0.46^{d}	20.5 ± 0.33^{e}
Hue (°H)	78.7 ± 0.43^{a}	79.6 ± 0.48^{b}	79.8 ± 0.53^{b}	79.7 ± 0.38^{b}	79.5 ± 0.15^{b}	79.2 ± 0.16^{b}

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45 ± 1.20^{d}	5.40 ± 0.93^{d}	5.05 ± 1.20^{cd}	4.85 ± 0.98^{bc}	4.55 ± 1.15^{ab}	4.33 ± 1.54^{a}
Aroma	3.35 ± 0.92^{a}	4.43 ± 1.36^{b}	4.83 ± 1.01^{b}	4.53 ± 1.22^{b}	4.73 ± 1.26^{b}	5.35 ± 0.95^{c}
Taste	3.33 ± 0.92^{a}	3.38 ± 0.90^{a}	4.10 ± 1.01^{b}	4.48 ± 1.26^{b}	4.93 ± 1.23^{c}	5.33 ± 0.76^{d}
Overall acceptance	3.35 ± 0.77^{a}	4.33 ± 1.05^{b}	4.80 ± 1.18^{c}	4.93 ± 1.21^{cd}	5.05 ± 1.20^{cd}	5.33 ± 0.97^{d}

Values are presented as mean$\pm$SD (n = 50). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

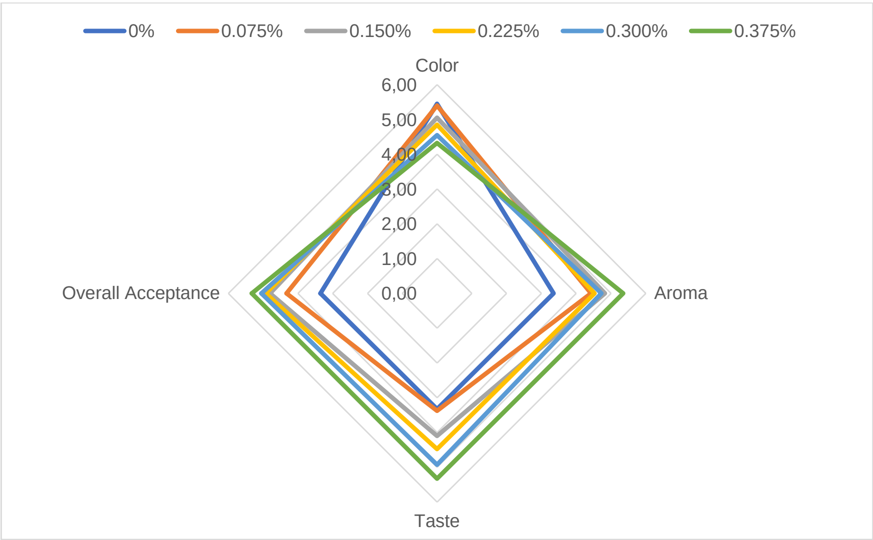

Figure 1. Spider web test result

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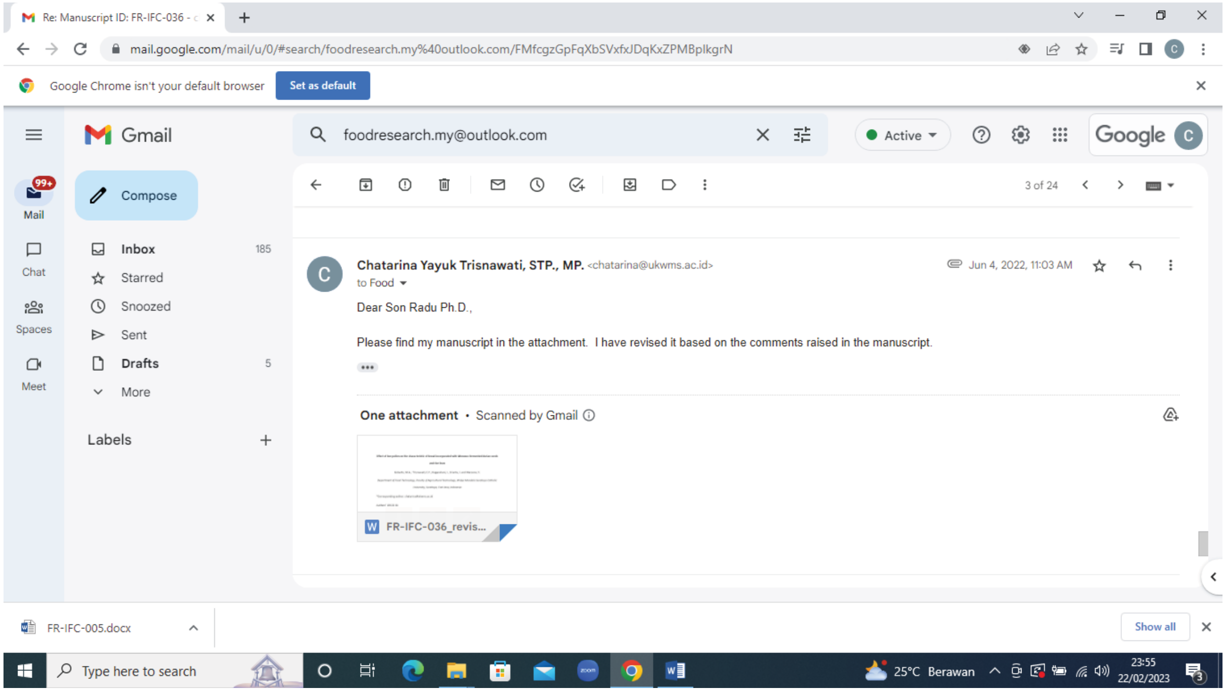
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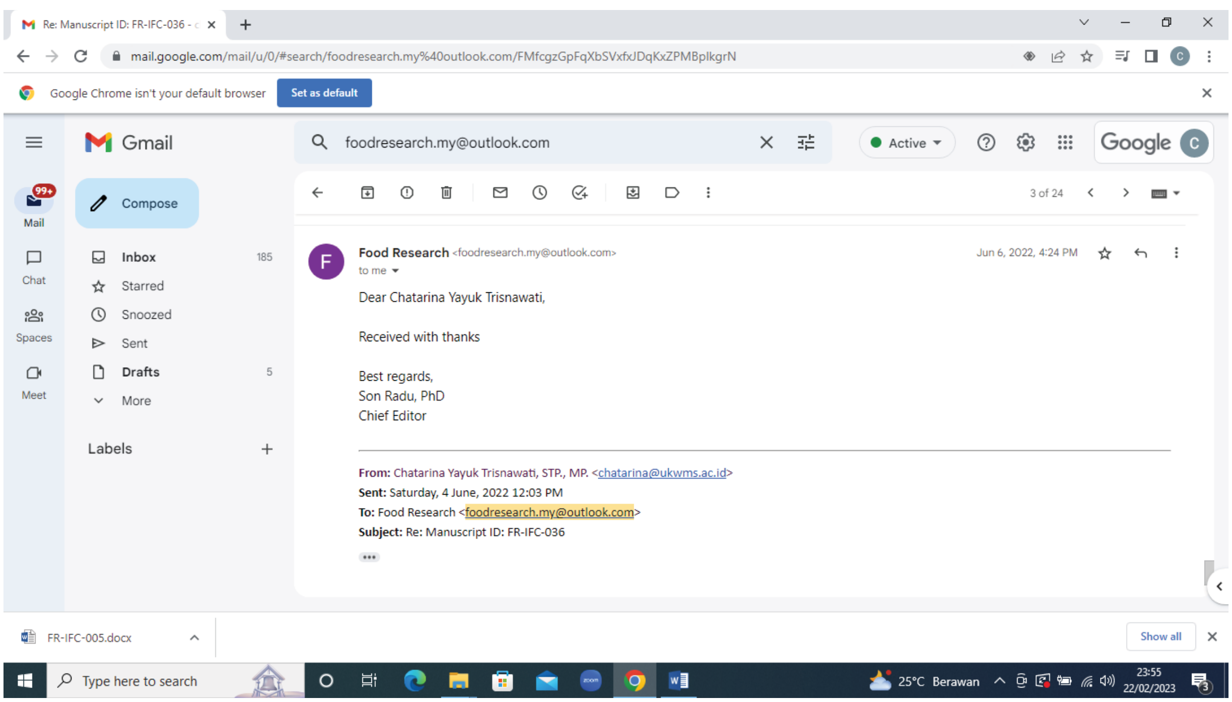
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Effect of bee pollen on the characteristic of bread incorporated with *Monasus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis

of Variance with α = 5%. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB (p>0.05). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance (p>0.05). The best treatment was obtained by using 0.375% bee pollen.

Keywords: Bread, *Monascus*-fermented durian seeds, Rice bran, Bee pollen

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production. (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and keton (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity

flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 min and followed by adding water. The mixing process then continued for 10 mins. Margarine and table salt was then added and the mixing process continued for 5 mins. The dough was fermented at 26°C for 30 mins. After the dough was shaped into a loaf the dough was proofed at 26°C for 90 mins. The bread was baked with the oven at 180°C for 30 mins then cooled at 26°C for 60 mins. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90°C for 10 mins [durian seeds: 5%

$Ca(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution and then washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through first drying process at 45°C for 40 mins. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 mins and cooled down at 26°C for 30 mins. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30$\pm$1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 h, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

2.3 Moisture content analysis

Moisture content determination was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)-weight loss (g)}} \times 100\%$$

2.4 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). These steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1\text{ (ml)-}V_2\text{ (ml)}}{\text{W (g)}}$$

2.5 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková *et al*., 2012).

2.6 Color analysis

Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm x 5 cm x 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture Content

According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease bread's springiness.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since it is brown in color and Maillard reaction which occurred during baking process.

Starowicz and Zieliński (2019) reported that free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L* value of food. Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value of b* was also influenced by the yellow pigment β-carotene in bee pollen.

3.3 Sensory evaluation

Table 6. showed that the preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web in Figure 1. test showed that 0.375% BP was the best concentration for bread incorporated with MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which derived from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen; B_1 : 0.075% bee pollen; B_2 : 0.150% bee pollen; B_3 : 0.225% bee pollen; B_4 : 0.300% bee pollen; B_5 : 0.375% bee pollen

Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Moisture Content (%)
B_0	40.29 ± 0.11^a
B_1	44.20 ± 0.07^b
B_2	47.34 ± 0.15^c
B_3	51.35 ± 0.16^d
B_4	54.23 ± 0.15^e
B_5	59.25 ± 0.16^f

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85 ± 0.03^c
B_1	3.97 ± 0.01^d
B_2	4.07 ± 0.06^e
B_3	3.81 ± 0.02^c
B_4	3.34 ± 0.06^b
B_5	3.23 ± 0.05^a

Values are presented as mean$\pm$SD (n = 4). Values with the same superscript within column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 ±7.920^{c}	769.631 ±10.910^{b}	665.708 ±5.590^{a}	833.009 ±10.450^{d}	1059.045 ±17.130^{e}	1254.505 ±15.140^{f}
Cohesiveness	0.643 ±0.010^{c}	0.662 ±0.010^{d}	0.677 ±0.010^{e}	0.646 ±0.010^{c}	0.620 ±0.010^{b}	0.596 ±0.010^{a}
Springiness	0.790 ±0.011^{c}	0.819 ±0.003^{d}	0.833 ±0.005^{e}	0.802 ±0.001^{d}	0.768 ±0.002^{b}	0.758 ±0.003^{a}

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2±0.25^{d}	67.6±0.39^{c}	66.8±0.99^{b}	66.5±0.20^{b}	65.7±0.55^{a}	65.3±0.17^{a}
Redness (a*)	3.2±0.13^{a}	3.2±0.10^{a}	3.3±0.18^{a}	3.4±0.13^{b}	3.6±0.10^{c}	3.8±0.03^{d}
Yellowness (b*)	15.9±0.13^{a}	17.4±0.28^{b}	18.2±0.22^{c}	18.6±0.45^{c}	19.5±0.45^{d}	20.1±0.32^{e}
Chroma (C)	16.2±0.15^{a}	17.7±0.28^{b}	18.5±0.22^{c}	18.9±0.45^{c}	19.8±0.46^{d}	20.5±0.33^{e}
Hue (^{o}H)	78.7±0.43^{a}	79.6±0.48^{b}	79.8±0.53^{b}	79.7±0.38^{b}	79.5±0.15^{b}	79.2±0.16^{b}

Values are presented as mean±SD (n = 4). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45 ± 1.20^{d}	5.40 ± 0.93^{d}	5.05 ± 1.20^{cd}	4.85 ± 0.98^{bc}	4.55 ± 1.15^{ab}	4.33 ± 1.54^{a}
Aroma	3.35 ± 0.92^{a}	4.43 ± 1.36^{b}	4.83 ± 1.01^{b}	4.53 ± 1.22^{b}	4.73 ± 1.26^{b}	5.35 ± 0.95^{c}
Taste	3.33 ± 0.92^{a}	3.38 ± 0.90^{a}	4.10 ± 1.01^{b}	4.48 ± 1.26^{b}	4.93 ± 1.23^{c}	5.33 ± 0.76^{d}
Overall acceptance	3.35 ± 0.77^{a}	4.33 ± 1.05^{b}	4.80 ± 1.18^{c}	4.93 ± 1.21^{cd}	5.05 ± 1.20^{cd}	5.33 ± 0.97^{d}

Values are presented as mean$\pm$SD (n = 50). Values with the same superscript within column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test (α = 0.05), B_0: 0% bee pollen, B_1: 0.075% bee pollen, B_2: 0.150% bee pollen, B_3: 0.225% bee pollen, B_4: 0.300% bee pollen, and B_5: 0.375% bee pollen

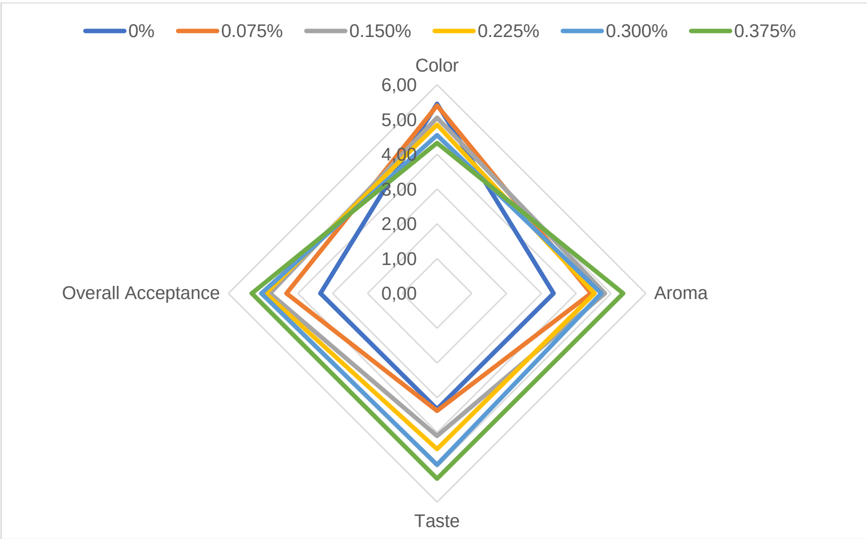

Figure 1. Spider web test result

10.

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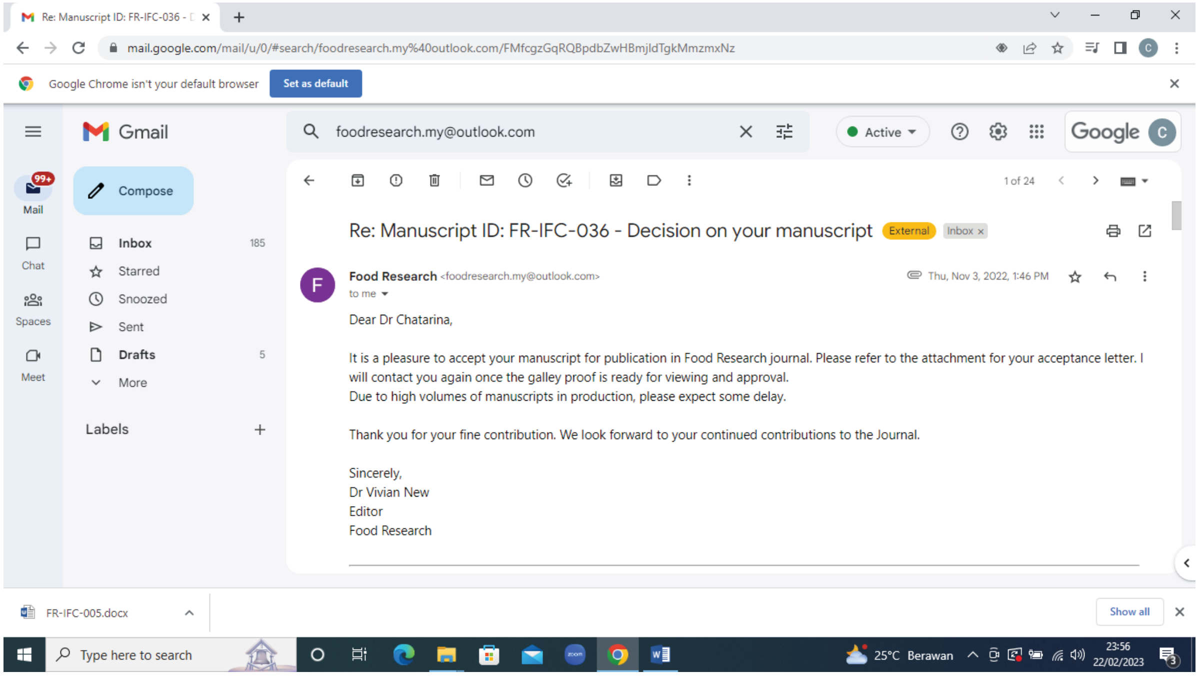

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Authors	:	Goberto, M.A., Trisnawati, C.Y., Nugerahani, I., Srianta, I. and Marsono, Y.

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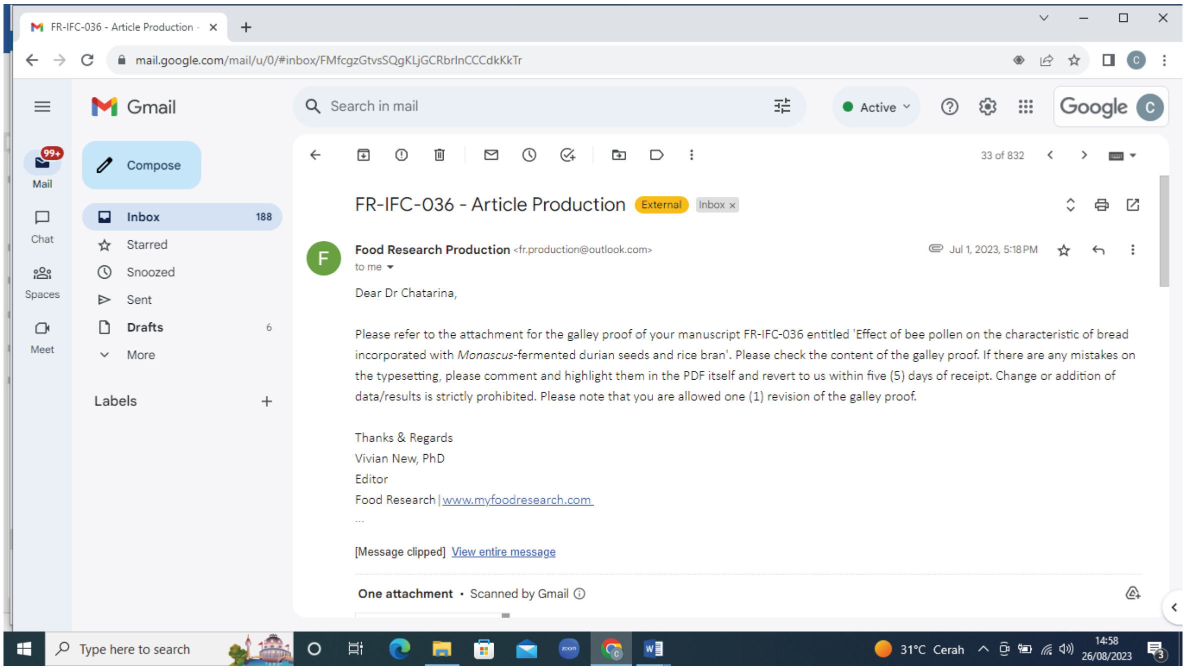

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FOOD RESEARCH

FULL PAPER

Effect of bee pollen on the characteristic of bread incorporated with *Monascus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis of Variance with $\alpha = 5\%$. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB ($p>0.05$). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance ($p>0.05$). The best treatment was obtained by using 0.375% bee pollen.

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and keton (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure

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culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 min and followed by adding water. The mixing process then continued for 10 mins. Margarine and table salt was then added and the mixing process continued for 5 mins. The dough was fermented at 26°C for 30 mins. After the dough was shaped into a loaf the dough was proofed at 26°C for 90 mins. The bread was baked with the oven at 180°C for 30 mins then cooled at 26°C for 60 mins. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90°C for 10 mins [durian seeds: 5% $Ca(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution and then washed with water. The durian seeds were then cut into 1 cm × 1 cm × 1 cm cubes and went through first drying process at 45°C for 40 mins. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 mins and cooled down at 26°C for 30 mins. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 hrs, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

2.4 Moisture content analysis

Moisture content determination was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)-weight loss (g)}} \times 100\%$$

2.5 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). These steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1\text{ (mL)-}V_2\text{ (mL)}}{\text{W (g)}}$$

2.6 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková *et al*., 2012).

2.7 Color analysis

Color analysis was carried out by measuring the

Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen, B_1 : 0.075% bee pollen, B_2 : 0.150% bee pollen, B_3 : 0.225% bee pollen, B_4 : 0.300% bee pollen, B_5 : 0.375% bee pollen.

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lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm × 5 cm × 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture content

According to Table 2, incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Bee pollen concentration	Moisture Content (%)
B_0	40.29 ± 0.11^a
B_1	44.20 ± 0.07^b
B_2	47.34 ± 0.15^c
B_3	51.35 ± 0.16^d
B_4	54.23 ± 0.15^e
B_5	59.25 ± 0.16^f

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease bread's springiness.

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85 ± 0.03^c
B_1	3.97 ± 0.01^d
B_2	4.07 ± 0.06^e
B_3	3.81 ± 0.02^c
B_4	3.34 ± 0.06^b
B_5	3.23 ± 0.05^a

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since it is brown in color and Maillard reaction which occurred during baking process. Starowicz and Zieliński (2019) reported that free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L* value of food. Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value of b* was also influenced by the yellow pigment β

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Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 ±7.920^c	769.631 ±10.910^b	665.708 ±5.590^a	833.009 ±10.450^d	1059.045 ±17.130^e	1254.505 ±15.140^f
Cohesiveness	0.643 ±0.010^c	0.662 ±0.010^d	0.677 ±0.010^e	0.646 ±0.010^c	0.620 ±0.010^b	0.596 ±0.010^a
Springiness	0.790 ±0.011^c	0.819 ±0.003^d	0.833 ±0.005^e	0.802 ±0.001^d	0.768 ±0.002^b	0.758 ±0.003^a

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2±0.25^d	67.6±0.39^c	66.8±0.99^b	66.5±0.20^b	65.7±0.55^a	65.3±0.17^a
Redness (a*)	3.2±0.13^a	3.2±0.10^a	3.3±0.18^a	3.4±0.13^b	3.6±0.10^c	3.8±0.03^d
Yellowness (b*)	15.9±0.13^a	17.4±0.28^b	18.2±0.22^c	18.6±0.45^c	19.5±0.45^d	20.1±0.32^e
Chroma (C)	16.2±0.15^a	17.7±0.28^b	18.5±0.22^c	18.9±0.45^c	19.8±0.46^d	20.5±0.33^e
Hue (^{o}H)	78.7±0.43^a	79.6±0.48^b	79.8±0.53^b	79.7±0.38^b	79.5±0.15^b	79.2±0.16^b

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

-carotene in bee pollen.

3.3 Sensory evaluation

Table 6 shows that the preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web in Figure 1. test showed that 0.375% BP was the best concentration for bread incorporated with MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which derived from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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AOAC (Association of Official Analytical Chemists).

Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45±1.20^d	5.40±0.93^d	5.05±1.20^{cd}	4.85±0.98^{bc}	4.55±1.15^{ab}	4.33±1.54^a
Aroma	3.35±0.92^a	4.43±1.36^b	4.83±1.01^b	4.53±1.22^b	4.73±1.26^b	5.35±0.95^c
Taste	3.33±0.92^a	3.38±0.90^a	4.10±1.01^b	4.48±1.26^b	4.93±1.23^c	5.33±0.76^d
Overall acceptance	3.35±0.77^a	4.33±1.05^b	4.80±1.18^c	4.93±1.21^{cd}	5.05±1.20^{cd}	5.33±0.97^d

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

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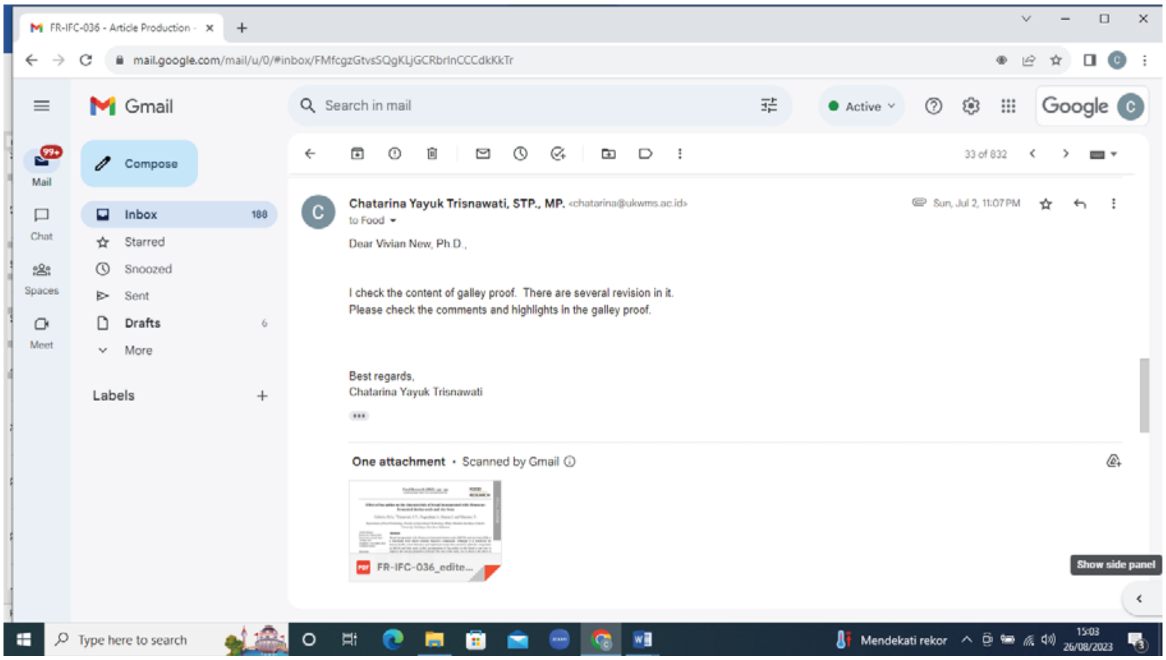
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Effect of bee pollen on the characteristic of bread incorporated with *Monascus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis of Variance with $\alpha = 5\%$. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB ($p>0.05$). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance ($p>0.05$). The best treatment was obtained by using 0.375% bee pollen.

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma from RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and keton (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure

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culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 min and followed by adding water. The mixing process then continued for 10 mins. Margarine and table salt was then added and the mixing process continued for 5 mins. The dough was fermented at 26°C for 30 mins. After the dough was shaped into a loaf the dough was proofed at 26°C for 90 mins. The bread was baked with the oven at 180°C for 30 mins then cooled at 26°C for 60 mins. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90°C for 10 mins [durian seeds: 5% $Ca(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution and then washed with water. The durian seeds were then cut into 1 cm × 1 cm × 1 cm cubes and went through first drying process at 45°C for 40 mins. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 mins and cooled down at 26°C for 30 mins. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 hrs, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.*, 2016).

2.4 Moisture content analysis

Moisture content determination was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)-weight loss (g)}} \times 100\%$$

2.5 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). These steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume}\left(\text{cm}^3/\text{g}\right) = \frac{V_1\ (\text{mL})\text{-}V_2\ (\text{mL})}{W\ (\text{g})}$$

2.6 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková *et al.*, 2012).

2.7 Color analysis

Color analysis was carried out by measuring the

Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

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Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which sized into 5 cm × 5 cm × 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture content

According to Table 2, incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Bee pollen concentration	Moisture Content (%)
B_0	40.29±0.11[a]
B_1	44.20±0.07[b]
B_2	47.34±0.15[c]
B_3	51.35±0.16[d]
B_4	54.23±0.15[e]
B_5	59.25±0.16[f]

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease bread's springiness.

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85±0.03[c]
B_1	3.97±0.01[d]
B_2	4.07±0.06[e]
B_3	3.81±0.02[c]
B_4	3.34±0.06[b]
B_5	3.23±0.05[a]

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since it is brown in color and Maillard reaction which occurred during baking process. Starowicz and Zieliński (2019) reported that free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L* value of food. Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value of b* was also influenced by the yellow pigment β

Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 ±7.920^{c}	769.631 ±10.910^{b}	665.708 ±5.590^{a}	833.009 ±10.450^{d}	1059.045 ±17.130^{e}	1254.505 ±15.140^{f}
Cohesiveness	0.643 ±0.010^{c}	0.662 ±0.010^{d}	0.677 ±0.010^{e}	0.646 ±0.010^{c}	0.620 ±0.010^{b}	0.596 ±0.010^{a}
Springiness	0.790 ±0.011^{c}	0.819 ±0.003^{d}	0.833 ±0.005^{e}	0.802 ±0.001^{d}	0.768 ±0.002^{b}	0.758 ±0.003^{a}

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2±0.25^{d}	67.6±0.39^{c}	66.8±0.99^{b}	66.5±0.20^{b}	65.7±0.55^{a}	65.3±0.17^{a}
Redness (a*)	3.2±0.13^{a}	3.2±0.10^{a}	3.3±0.18^{a}	3.4±0.13^{b}	3.6±0.10^{c}	3.8±0.03^{d}
Yellowness (b*)	15.9±0.13^{a}	17.4±0.28^{b}	18.2±0.22^{c}	18.6±0.45^{c}	19.5±0.45^{d}	20.1±0.32^{e}
Chroma (C)	16.2±0.15^{a}	17.7±0.28^{b}	18.5±0.22^{c}	18.9±0.45^{c}	19.8±0.46^{d}	20.5±0.33^{e}
Hue (°H)	78.7±0.43^{a}	79.6±0.48^{b}	79.8±0.53^{b}	79.7±0.38^{b}	79.5±0.15^{b}	79.2±0.16^{b}

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

-carotene in bee pollen.

3.3 Sensory evaluation

Table 6 shows that the preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web in Figure 1. test showed that 0.375% BP was the best concentration for bread incorporated with MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which derived from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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AOAC (Association of Official Analytical Chemists).

Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45±1.20^{d}	5.40±0.93^{d}	5.05±1.20^{cd}	4.85±0.98^{bc}	4.55±1.15^{ab}	4.33±1.54^{a}
Aroma	3.35±0.92^{a}	4.43±1.36^{b}	4.83±1.01^{b}	4.53±1.22^{b}	4.73±1.26^{b}	5.35±0.95^{c}
Taste	3.33±0.92^{a}	3.38±0.90^{a}	4.10±1.01^{b}	4.48±1.26^{b}	4.93±1.23^{c}	5.33±0.76^{d}
Overall acceptance	3.35±0.77^{a}	4.33±1.05^{b}	4.80±1.18^{c}	4.93±1.21^{cd}	5.05±1.20^{cd}	5.33±0.97^{d}

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

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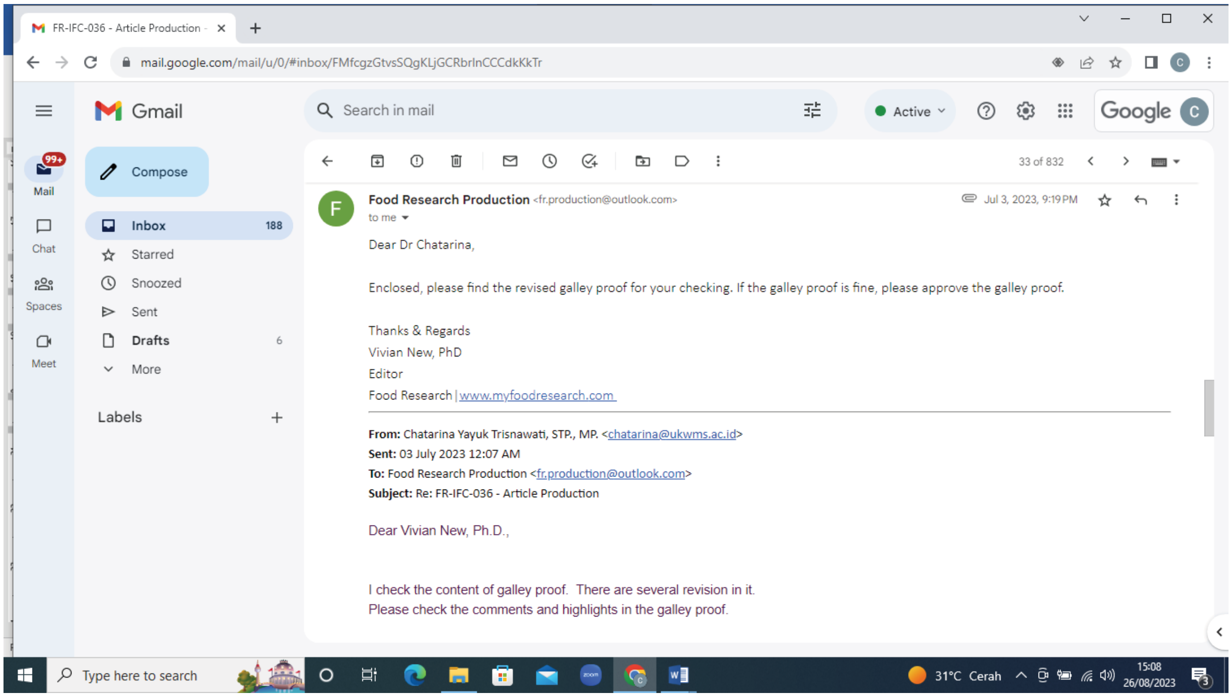

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FOOD RESEARCH

FULL PAPER

Effect of bee pollen on the characteristic of bread incorporated with *Monascus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis of Variance with $\alpha = 5\%$. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB ($p>0.05$). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance ($p>0.05$). The best treatment was obtained by using 0.375% bee pollen.

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to meet consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid and which produce bitter and astringent taste (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma caused by RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and keton (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic Surabaya Catholic University. Materials that were used for producing MFDS flour were Petruk durian seeds,

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pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 min and followed by adding water. The mixing process then continued for 10 mins. Margarine and table salt was then added and the mixing process continued for 5 mins. The dough was fermented at 26°C for 30 mins. After the dough was shaped into a loaf the dough was proofed at 26°C for 90 mins. The bread was baked in the oven at 180°C for 30 mins then cooled at 26°C for 60 mins. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with durian seeds sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90°C for 10 mins [durian seeds: 5% Ca $(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution, then washed with water. The durian seeds were then cut into 1 cm × 1 cm × 1 cm cubes and went through first drying process at 45°C for 40 mins. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 mins and cooled down at 26°C for 30 mins. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 hrs, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

2.4 Moisture content analysis

Moisture content determination was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g) - weight loss (g)}}{\text{sample weight (g)}} \times 100\%$$

2.5 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). These steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1\ (\text{mL}) - V_2\ (\text{mL})}{W\ (\text{g})}$$

2.6 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková *et al*., 2012).

2.7 Color analysis

Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen, B_1 : 0.075% bee pollen, B_2 : 0.150% bee pollen, B_3 : 0.225% bee pollen, B_4 : 0.300% bee pollen, B_5 : 0.375% bee pollen.

DOI

Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which were cut into 5 cm × 5 cm × 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

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Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture content

According to Table 2, incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). The bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

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B_4	54.23±0.15[e]
B_5	59.25±0.16[f]

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread's hardness and decrease bread's springiness.

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85±0.03[c]
B_1	3.97±0.01[d]
B_2	4.07±0.06[e]
B_3	3.81±0.02[c]
B_4	3.34±0.06[b]
B_5	3.23±0.05[a]

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since its color is brown and Maillard reaction which occurred during baking process. Starowicz and Zieliński (2019) reported that free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L* value of food. Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value.

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Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 ±7.920^c	769.631 ±10.910^b	665.708 ±5.590^a	833.009 ±10.450^d	1059.045 ±17.130^e	1254.505 ±15.140^f
Cohesiveness	0.643 ±0.010^c	0.662 ±0.010^d	0.677 ±0.010^e	0.646 ±0.010^c	0.620 ±0.010^b	0.596 ±0.010^a
Springiness	0.790 ±0.011^c	0.819 ±0.003^d	0.833 ±0.005^e	0.802 ±0.001^d	0.768 ±0.002^b	0.758 ±0.003^a

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2±0.25^d	67.6±0.39^c	66.8±0.99^b	66.5±0.20^b	65.7±0.55^a	65.3±0.17^a
Redness (a*)	3.2±0.13^a	3.2±0.10^a	3.3±0.18^a	3.4±0.13^b	3.6±0.10^c	3.8±0.03^d
Yellowness (b*)	15.9±0.13^a	17.4±0.28^b	18.2±0.22^c	18.6±0.45^c	19.5±0.45^d	20.1±0.32^e
Chroma (C)	16.2±0.15^a	17.7±0.28^b	18.5±0.22^c	18.9±0.45^c	19.8±0.46^d	20.5±0.33^e
Hue (^{o}H)	78.7±0.43^a	79.6±0.48^b	79.8±0.53^b	79.7±0.38^b	79.5±0.15^b	79.2±0.16^b

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

Value of b* was also influenced by the yellow pigment β -carotene in bee pollen.

3.3 Sensory evaluation

Table 6 shows that the preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web in Figure 1. test showed that 0.375% BP was the best concentration for bread incorporated with MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which came from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

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AOAC (Association of Official Analytical Chemists).

Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45±1.20^d	5.40±0.93^d	5.05±1.20^{cd}	4.85±0.98^{bc}	4.55±1.15^{ab}	4.33±1.54^a
Aroma	3.35±0.92^a	4.43±1.36^b	4.83±1.01^b	4.53±1.22^b	4.73±1.26^b	5.35±0.95^c
Taste	3.33±0.92^a	3.38±0.90^a	4.10±1.01^b	4.48±1.26^b	4.93±1.23^c	5.33±0.76^d
Overall acceptance	3.35±0.77^a	4.33±1.05^b	4.80±1.18^c	4.93±1.21^{cd}	5.05±1.20^{cd}	5.33±0.97^d

Values are presented as mean±SD (n = 50). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

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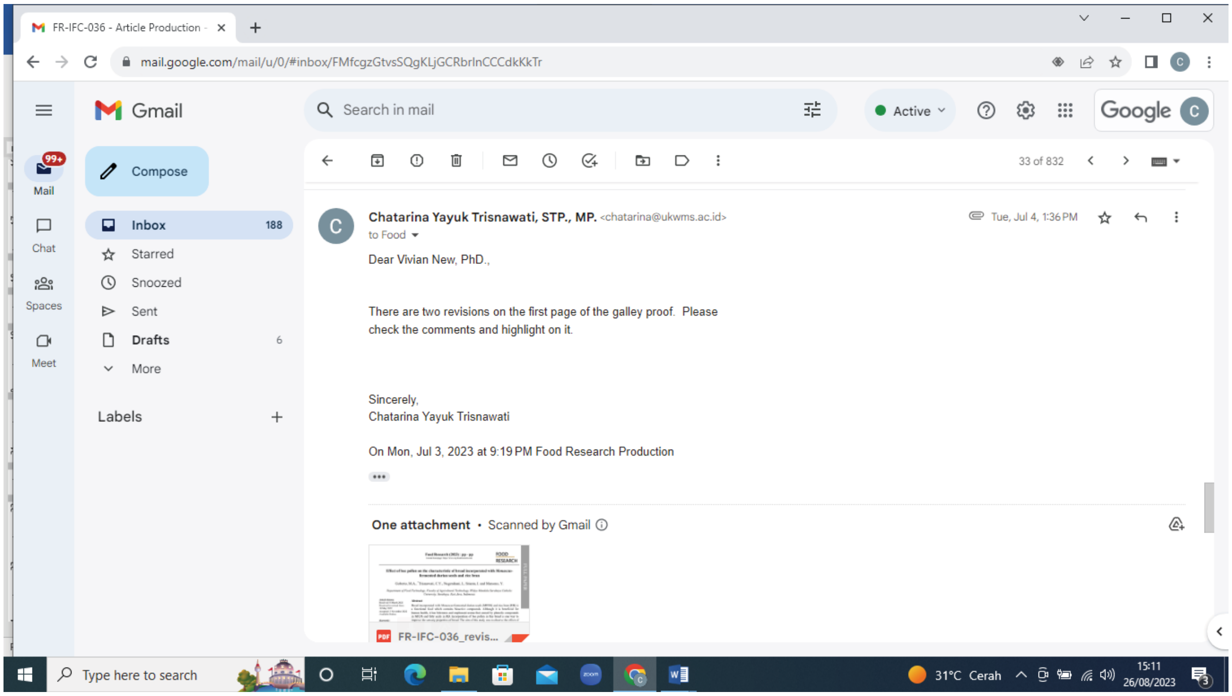
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Effect of bee pollen on the characteristic of bread incorporated with *Monascus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis of Variance with $\alpha = 5\%$. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB ($p>0.05$). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance ($p>0.05$). The best treatment was obtained by using 0.375% bee pollen.

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to meet consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid and which produce bitter and astringent taste (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma caused by RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and keton (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic Surabaya Catholic University. Materials that were used for producing MFDS flour were Petruk durian seeds,

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pure culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 min and followed by adding water. The mixing process then continued for 10 mins. Margarine and table salt was then added and the mixing process continued for 5 mins. The dough was fermented at 26°C for 30 mins. After the dough was shaped into a loaf the dough was proofed at 26°C for 90 mins. The bread was baked in the oven at 180°C for 30 mins then cooled at 26°C for 60 mins. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with durian seeds sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90°C for 10 mins [durian seeds: 5% Ca $(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution, then washed with water. The durian seeds were then cut into 1 cm × 1 cm × 1 cm cubes and went through first drying process at 45°C for 40 mins. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 mins and cooled down at 26°C for 30 mins. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 hrs, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

2.4 Moisture content analysis

Moisture content determination was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g) - weight loss (g)}}{\text{sample weight (g)}} \times 100\%$$

2.5 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). These steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1\ (\text{mL}) - V_2\ (\text{mL})}{W\ (\text{g})}$$

2.6 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková *et al*., 2012).

2.7 Color analysis

Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen, B_1 : 0.075% bee pollen, B_2 : 0.150% bee pollen, B_3 : 0.225% bee pollen, B_4 : 0.300% bee pollen, B_5 : 0.375% bee pollen.

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Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which were cut into 5 cm × 5 cm × 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture content

According to Table 2, incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). The bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Bee pollen concentration	Moisture Content (%)
B_0	$40.29{\pm}0.11^a$
B_1	$44.20{\pm}0.07^b$
B_2	$47.34{\pm}0.15^c$
B_3	$51.35{\pm}0.16^d$
B_4	$54.23{\pm}0.15^e$
B_5	$59.25{\pm}0.16^f$

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread's hardness and decrease bread's springiness.

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Bee pollen concentration	Specific volume (cm^3/g)
B_0	$3.85{\pm}0.03^c$
B_1	$3.97{\pm}0.01^d$
B_2	$4.07{\pm}0.06^e$
B_3	$3.81{\pm}0.02^c$
B_4	$3.34{\pm}0.06^b$
B_5	$3.23{\pm}0.05^a$

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since its color is brown and Maillard reaction which occurred during baking process. Starowicz and Zieliński (2019) reported that free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L* value of food. Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value.

Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 ±7.920^{c}	769.631 ±10.910^{b}	665.708 ±5.590^{a}	833.009 ±10.450^{d}	1059.045 ±17.130^{e}	1254.505 ±15.140^{f}
Cohesiveness	0.643 ±0.010^{c}	0.662 ±0.010^{d}	0.677 ±0.010^{e}	0.646 ±0.010^{c}	0.620 ±0.010^{b}	0.596 ±0.010^{a}
Springiness	0.790 ±0.011^{c}	0.819 ±0.003^{d}	0.833 ±0.005^{e}	0.802 ±0.001^{d}	0.768 ±0.002^{b}	0.758 ±0.003^{a}

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2±0.25^{d}	67.6±0.39^{c}	66.8±0.99^{b}	66.5±0.20^{b}	65.7±0.55^{a}	65.3±0.17^{a}
Redness (a*)	3.2±0.13^{a}	3.2±0.10^{a}	3.3±0.18^{a}	3.4±0.13^{b}	3.6±0.10^{c}	3.8±0.03^{d}
Yellowness (b*)	15.9±0.13^{a}	17.4±0.28^{b}	18.2±0.22^{c}	18.6±0.45^{c}	19.5±0.45^{d}	20.1±0.32^{e}
Chroma (C)	16.2±0.15^{a}	17.7±0.28^{b}	18.5±0.22^{c}	18.9±0.45^{c}	19.8±0.46^{d}	20.5±0.33^{e}
Hue (^{o}H)	78.7±0.43^{a}	79.6±0.48^{b}	79.8±0.53^{b}	79.7±0.38^{b}	79.5±0.15^{b}	79.2±0.16^{b}

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

Value of b* was also influenced by the yellow pigment β -carotene in bee pollen.

3.3 Sensory evaluation

Table 6 shows that the preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web in Figure 1. test showed that 0.375% BP was the best concentration for bread incorporated with MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which came from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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AOAC (Association of Official Analytical Chemists).

Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45±1.20^{d}	5.40±0.93^{d}	5.05±1.20^{cd}	4.85±0.98^{bc}	4.55±1.15^{ab}	4.33±1.54^{a}
Aroma	3.35±0.92^{a}	4.43±1.36^{b}	4.83±1.01^{b}	4.53±1.22^{b}	4.73±1.26^{b}	5.35±0.95^{c}
Taste	3.33±0.92^{a}	3.38±0.90^{a}	4.10±1.01^{b}	4.48±1.26^{b}	4.93±1.23^{c}	5.33±0.76^{d}
Overall acceptance	3.35±0.77^{a}	4.33±1.05^{b}	4.80±1.18^{c}	4.93±1.21^{cd}	5.05±1.20^{cd}	5.33±0.97^{d}

Values are presented as mean±SD (n = 50). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

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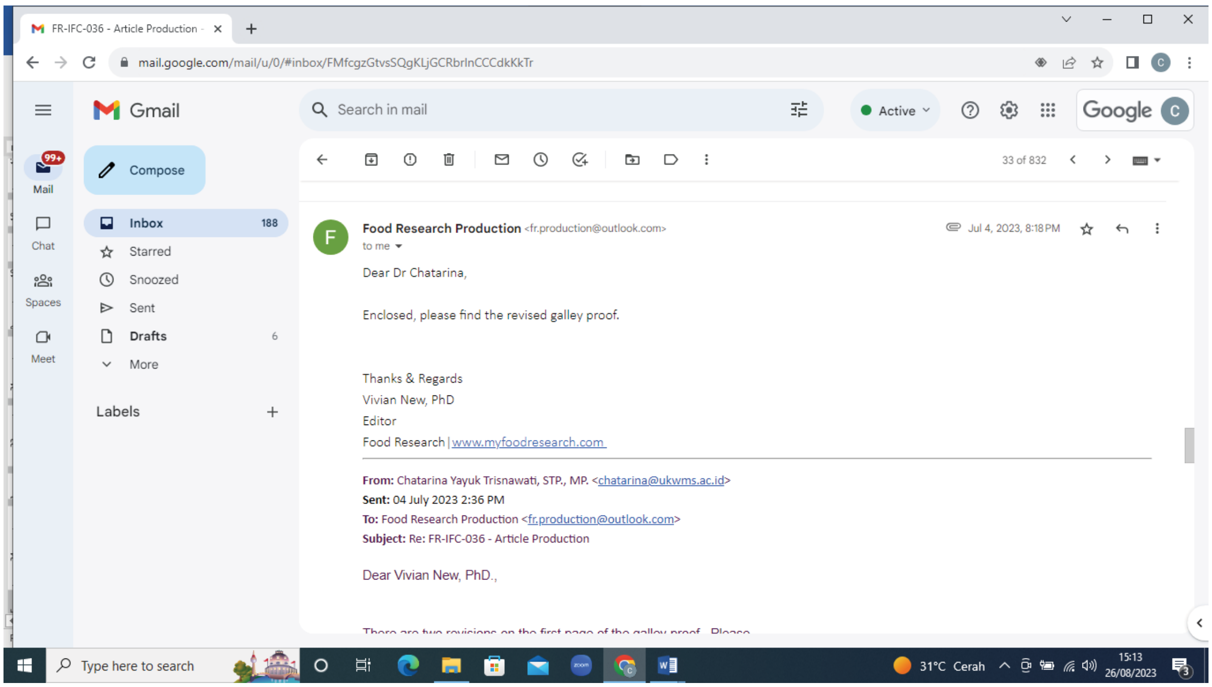

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FOOD RESEARCH

FULL PAPER

Effect of bee pollen on the characteristic of bread incorporated with *Monascus*-fermented durian seeds and rice bran

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Abstract

Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was to observe the effect of different bee pollen concentrations on the physicochemical and sensory properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six levels of treatment starting from 0, 0.075, 0.150, 0.225, 0.300 and 0.375%. Data were analyzed by Analysis of Variance with $\alpha = 5\%$. The results indicated that difference in bee pollen concentration significantly affected on physicochemical and sensory properties of bread incorporated with MFDS and RB ($p>0.05$). Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall acceptance ($p>0.05$). The best treatment was obtained by using 0.375% bee pollen.

1. Introduction

The development of functional bakery products had been widely studied among food scientists as an approach to meet consumer's demand for baked products with extra health benefits. Trisnawati *et al.* (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented durian seeds (MFDS) into bread. Rice bran contains γ-orizanol, γ-tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.,* 2013; Sivamaruthi *et al.,* 2018). MFDS contains monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production (Nugerahani *et al.,* 2017; Faroukh and Baumgärtel, 2019).

Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as tannin and alkaloid which produce bitter and astringent taste (Reginio *et al.,* 2016; Hasim *et al.,* 2019).

One way to cover the unpleasant taste and aroma caused by RB and MFDS is by incorporating bee pollen. There are several volatile compounds in bee pollen such as esters, hydrocarbon, aldehyde, terpenoid, and keton (Neto *et al.,* 2017). Incorporation of bee pollen also may increase furan production, such as furfural and pyrazine in the final product. These compounds produce caramel, floral and fruity flavors (Conte *et al.,* 2020). Unsaturated fatty acid, phytosterol and phospholipid in bee pollen can increase hypoglycemic activity (Komosinska-Vassev *et al.,* 2015). The objective of this study was to observe the effect of bee pollen incorporation on the characteristic of bread incorporated with MFDS and RB.

2. Materials and methods

2.1 Materials

Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Surabaya Catholic University. Materials that were used for producing MFDS flour were Petruk durian seeds, pure

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culture of *Monascus purpureus* M9, $Ca(OH)_2$, distilled water and potato dextrose agar (Merck 1.10130.0500).

2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran

Sample preparation began by mixing all of the dry ingredients except table salt for 1 min and followed by adding water. The mixing process then continued for 10 mins. Margarine and table salt was then added and the mixing process continued for 5 mins. The dough was fermented at 26°C for 30 mins. After the dough was shaped into a loaf the dough was proofed at 26°C for 90 mins. The bread was baked in the oven at 180°C for 30 mins then cooled at 26°C for 60 mins. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels of bee pollen concentration.

2.3 Monascus-fermented durian seeds preparation

Preparation of MFDS flour began with durian seeds sortation. Durian seeds then washed with water. After that, the durian seeds were boiled with 5% $Ca(OH)_2$ solution at 85-90°C for 10 mins [durian seeds: 5% Ca $(OH)_2$ solution = 1:1 (w/v)]. The durian seeds were removed from the $Ca(OH)_2$ solution, then washed with water. The durian seeds were then cut into 1 cm × 1 cm × 1 cm cubes and went through first drying process at 45°C for 40 mins. The durian seeds were then weighed into 50 g. After that, durian seeds were sterilized at 121°C, 15 lbs/inch^2 for 10 mins and cooled down at 26°C for 30 mins. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at 45°C for 24 hrs, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.,* 2016).

2.4 Moisture content analysis

Moisture content determination was carried out using thermogravimetric method according to AOAC 925.10 (1990). The moisture content of bread incorporated with MFDS and RB was determined using following equation:

$$\text{Moisture content (\%)} = \frac{\text{sample weight (g) - weight loss (g)}}{\text{sample weight (g)}} \times 100\%$$

2.5 Specific volume

Specific volume determination was carried out using seed displacement method according to Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds which were above the container rim were then removed using straight ruler. All the foxtail millet seeds in the container were then poured into a measuring cylinder to measure the volume of the container (V_1). These steps were then repeated except that a loaf of bread sample was already inside the container before it was filled with seeds to obtain V_2.

$$\text{Specific volume } \left(\text{cm}^3/\text{g}\right) = \frac{V_1\text{ (mL)-}V_2\text{ (mL)}}{W\text{ (g)}}$$

2.6 Texture analysis

Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential compression events (probe: P36/R, pre-test speed: 5 mm s^{-1}, test speed: 1.5 mm s^{-1}, post-test speed 10 mm s^{-1}, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

Hardness was defined as force that needed to achieve deformation during first compression. Cohesiveness was defined as ratio of the positive first area of the second peak to the first peak. Springiness was defined as ability of the bread to recover in height during the time elapsed between end of first compression and start of the second compression cycle (Dvořáková *et al*., 2012).

2.7 Color analysis

Table 1. Formulation of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Ingredients/g	B_0	B_1	B_2	B_3	B_4	B_5
Bread flour	179.85	179.85	179.85	179.85	179.85	179.85
Mineral water	124	124	124	124	124	124
Rice bran flour	20	20	20	20	20	20
Granulated sugar	10	10	10	10	10	10
Margarine	8	8	8	8	8	8
Full cream milk powder	4	4	4	4	4	4
Instant dry yeast	3	3	3	3	3	3
Table salt	2	2	2	2	2	2
Bread improver	0.6	0.6	0.6	0.6	0.6	0.6
MFDS flour	0.15	0.15	0.15	0.15	0.15	0.15
Bee pollen	0	0.075	0.150	0.225	0.300	0.375

B_0 : 0% bee pollen, B_1 : 0.075% bee pollen, B_2 : 0.150% bee pollen, B_3 : 0.225% bee pollen, B_4 : 0.300% bee pollen, B_5 : 0.375% bee pollen.

DOI

Color analysis was carried out by measuring the lightness (L*), redness (a*), yellowness (b*), chroma (*C) and hue (°H) of the crumb of bread incorporated MFDS and RB with color reader (Minolta CR-10 Chroma Meter).

2.8 Sensory evaluation

Sensory evaluation was carried out with 50 untrained panelists. Each panelist was asked to evaluate each parameter using a 7-point hedonic scale (Stone and Sidel, 2004). Scores were assigned in a range 1-7 (1 = extremely dislike; 2 = dislike; 3 = slightly dislike; 4 = neither like nor dislike; 5 = slightly like; 6 = like; 7 = extremely like). Each panelist received four slices of bread incorporated with *Monascus*-fermented durian seed and rice bran which were cut into 5 cm × 5 cm × 1 cm. The tested parameters consisted of preference score for color, aroma, taste, and overall acceptance.

2.9 Statistical analysis

Data were generated in triplicate and subjected to Analysis of Variance (ANOVA) with α = 5% and followed by Duncan's Multiple Range Test (DMRT) with α = 5% using SPSS software. Data collected from sensory evaluation were also subjected to spider-web test using Microsoft Excel 2013.

3. Results and discussion

3.1 Moisture content

According to Table 2, incorporation of bee pollen to bread with MFDS and RB resulted in moisture content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice bran contains 7-11% fiber (Henderson *et al.,* 2012). Ability of fiber to bind with water then resulted in increased moisture content of bread (Sangle *et al.,* 2017). Bee pollen contains glucose, fructose and sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*, 2013). The bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were hydrophilic amino acid (Taha *et al.,* 2017). Conte *et al.* (2020) also reported that moisture content of gluten free bread increased as bee pollen concentration increased.

Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Bee pollen concentration	Moisture Content (%)
B_0	40.29 ± 0.11^a
B_1	44.20 ± 0.07^b
B_2	47.34 ± 0.15^c
B_3	51.35 ± 0.16^d
B_4	54.23 ± 0.15^e
B_5	59.25 ± 0.16^f

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

3.2 Physical properties

Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due to additional sugar from bee pollen which are useful CO_2 production by yeast. The CO_2 pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO_2 gas also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a decrease in specific volume due to competition in binding water between the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker and could not retain CO_2 as much as bread with 0-0.15%. Lower retention of CO_2 also resulted in bread with thicker gluten matrix and lower viscoelasticity hence increase bread's hardness and decrease bread's springiness.

Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Bee pollen concentration	Specific volume (cm^3/g)
B_0	3.85 ± 0.03^c
B_1	3.97 ± 0.01^d
B_2	4.07 ± 0.06^e
B_3	3.81 ± 0.02^c
B_4	3.34 ± 0.06^b
B_5	3.23 ± 0.05^a

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of rice bran flour since its color is brown and Maillard reaction which occurred during baking process. Starowicz and Zieliński (2019) reported that free amine groups in protein and carbonyl groups in sugars went through Maillard reaction to produce melanoidin which reduce L* value of food. Brown's base colors are grounded with red and yellow which resulted in increased of a* and b* value.

DOI

Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Textural properties	B_0	B_1	B_2	B_3	B_4	B_5
Hardness (g)	790.452 ±7.920^c	769.631 ±10.910^b	665.708 ±5.590^a	833.009 ±10.450^d	1059.045 ±17.130^e	1254.505 ±15.140^f
Cohesiveness	0.643 ±0.010^c	0.662 ±0.010^d	0.677 ±0.010^e	0.646 ±0.010^c	0.620 ±0.010^b	0.596 ±0.010^a
Springiness	0.790 ±0.011^c	0.819 ±0.003^d	0.833 ±0.005^e	0.802 ±0.001^d	0.768 ±0.002^b	0.758 ±0.003^a

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Color evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Lightness (L*)	69.2±0.25^d	67.6±0.39^c	66.8±0.99^b	66.5±0.20^b	65.7±0.55^a	65.3±0.17^a
Redness (a*)	3.2±0.13^a	3.2±0.10^a	3.3±0.18^a	3.4±0.13^b	3.6±0.10^c	3.8±0.03^d
Yellowness (b*)	15.9±0.13^a	17.4±0.28^b	18.2±0.22^c	18.6±0.45^c	19.5±0.45^d	20.1±0.32^e
Chroma (C)	16.2±0.15^a	17.7±0.28^b	18.5±0.22^c	18.9±0.45^c	19.8±0.46^d	20.5±0.33^e
Hue (^{o}H)	78.7±0.43^a	79.6±0.48^b	79.8±0.53^b	79.7±0.38^b	79.5±0.15^b	79.2±0.16^b

Values are presented as mean±SD (n = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

Value of b* was also influenced by the yellow pigment β -carotene in bee pollen.

3.3 Sensory evaluation

Table 6 shows that the preference score for color of the bread samples was statistically decreased while preference score of taste, aroma and overall acceptability were statistically increased as BP concentration increased. Spider web in Figure 1. test showed that 0.375% BP was the best concentration for bread incorporated with MFDS and RB even though the color was the least favorable.

Bee pollen consisted of several volatile compounds which comprised of hydrocarbon, esters, terpenoid and alcohol which produced floral and fruity aroma (Neto *et al.,* 2017). Increased in bee pollen concentration also resulted in more Maillard reaction product resulted such as pyrazine that produced nutty and roasted aroma; furan that produced sweet and caramel aroma; acetylpyridine that produced cracker-malty aroma; and pyrol that produced nutty aroma (van Boekel, 2006). Those volatile compounds together were able to tone down the rancid aroma, which came from the rice bran.

4. Conclusion

The most suitable bee pollen concentration for bread incorporated with MFDS and RB is 0.375% according to the sensory evaluation. The overall acceptance score of this bread represented that panelist slightly like the bread. This study showed that bee pollen has the potential to improve taste and aroma of bread incorporated with MFDS and RB. Further study to improve specific volume and textural properties of this bread is suggested.

Conflict of interest

The authors declare no conflict of interest.

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Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian seeds and rice bran.

Sensory evaluation	B_0	B_1	B_2	B_3	B_4	B_5
Color	5.45±1.20^d	5.40±0.93^d	5.05±1.20^{cd}	4.85±0.98^{bc}	4.55±1.15^{ab}	4.33±1.54^a
Aroma	3.35±0.92^a	4.43±1.36^b	4.83±1.01^b	4.53±1.22^b	4.73±1.26^b	5.35±0.95^c
Taste	3.33±0.92^a	3.38±0.90^a	4.10±1.01^b	4.48±1.26^b	4.93±1.23^c	5.33±0.76^d
Overall acceptance	3.35±0.77^a	4.33±1.05^b	4.80±1.18^c	4.93±1.21^{cd}	5.05±1.20^{cd}	5.33±0.97^d

Values are presented as mean±SD (n = 50). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test (α = 0.05). B0: 0% bee pollen, B1: 0.075% bee pollen, B2: 0.150% bee pollen, B3: 0.225% bee pollen, B4: 0.300% bee pollen, B5: 0.375% bee pollen.

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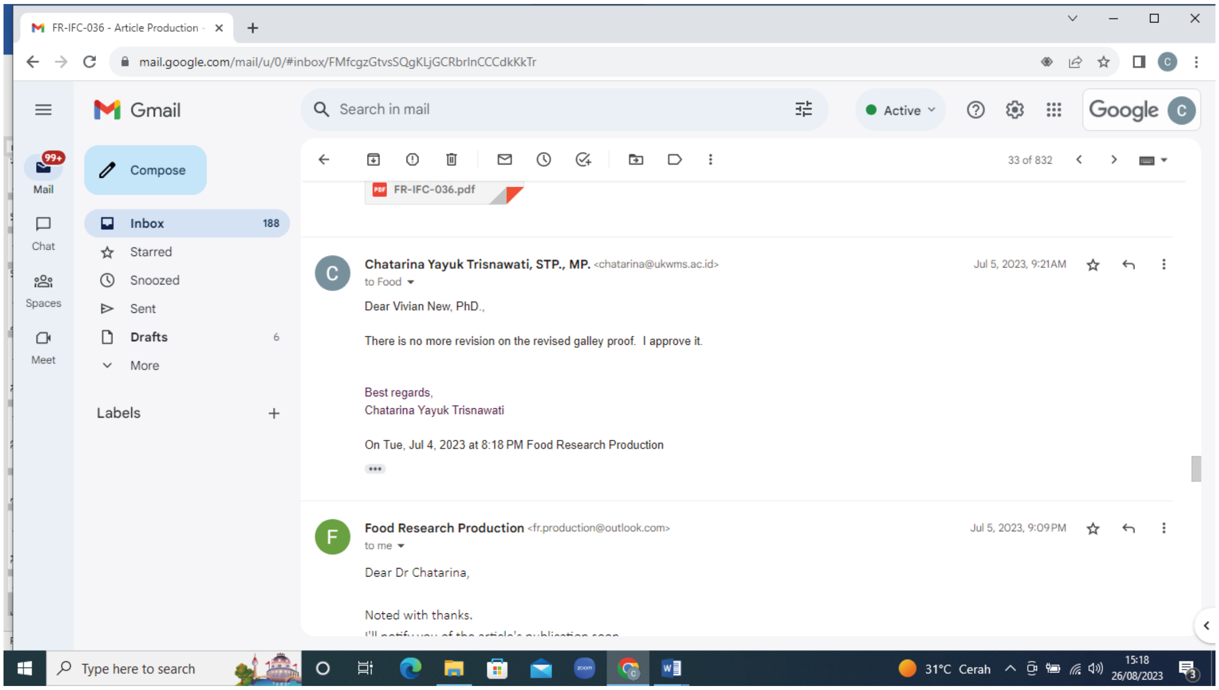
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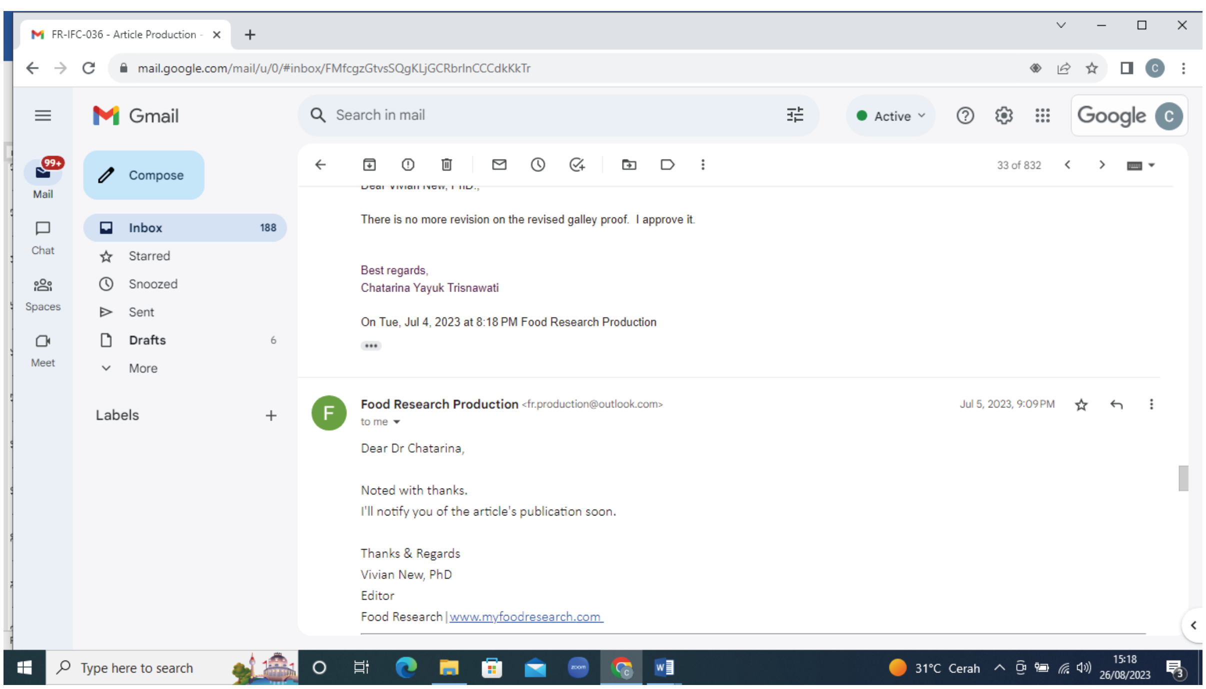
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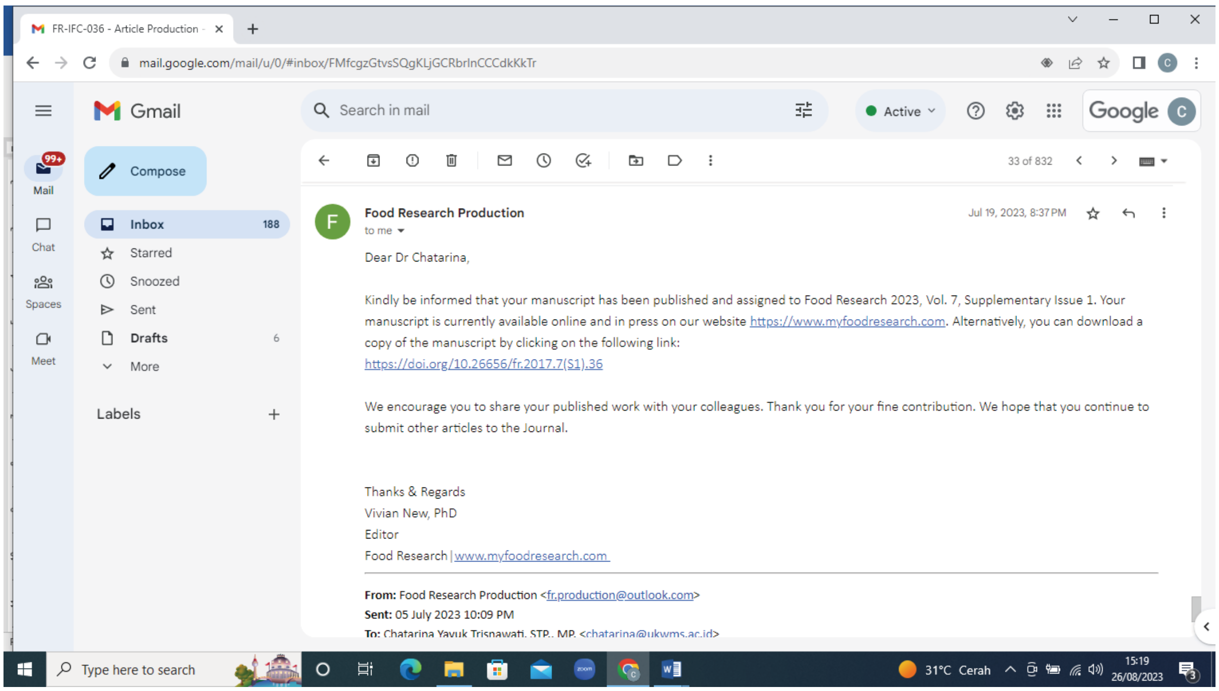

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