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

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13

14 **Abstract**

15 Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food
16 which contains bioactive compounds. MFDS contains monacolin K which is able reduce cholesterol while
17 RB contains non-dietary fiber, oryzanol, and tocotrienol which can prevent hyperglycemic. Although it is
18 beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds
19 in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory
20 properties of bread since it contains volatile compounds with pleasant taste and aroma such as furan,
21 furfural, and pyrazine. The aim of this study was to observe the effect of different bee pollen

22 concentrations on the sensory properties of bread incorporated with MFDS and RB. This study used
23 Randomized Block Design with six levels of treatment starting from 0%; 0.075%; 0.150%; 0.225%; 0.300%;
24 and 0.375%. Data were analyzed by Analysis of Variance with $\alpha = 5\%$. The result of this research showed
25 that different concentration of bee pollen significantly affected the sensory properties of bread
26 incorporated with MFDS and RB. The preference score for color ranged from 4.33 to 5.45; aroma ranged
27 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
28 bee pollen concentration increased, preference score for aroma, taste, and overall acceptance increased
29 and preference score for color decreased. The best treatment was obtained by using 0,375% bee pollen.

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

31

32 1. Introduction

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

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

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

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

68 2. Materials and methods

69 2.1 Materials

70 Materials that used for making bread incorporated with *Monascus*-fermented durian seeds and
71 rice bran were “Cakra Kembar” high-protein wheat flour, “dr.Liem” rice bran flour, “Fermipan” instant dry
72 yeast, “Dancow” instant full cream milk powder, “Gulaku” granulated sugar, “Bakerine” bread improver,
73 “Cap Kapal” table salt, “Aqua” mineral water, “Blueband” margarine which were purchased from local
74 distributor, “Mirah Delima” multiflora bee pollen which was purchased from Mirah Delima Bee Farm and
75 also *Monascus*-fermented durian seeds powder which was produced in Laboratory of Food Industrial
76 Microbiology, Widya Mandala Catholic University Surabaya. Materials that used for making *Monascus*-
77 fermented durian seeds were Petruk durian seeds, pure culture of *Monascus purpureus* M9, Ca(OH)₂,
78 aquadest and potato dextrose agar (Merck 1.10130.0500).

79 2.2 Preparation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

80 Process of making bread incorporated with *Monascus*-fermented durian seeds and rice bran
81 consisted of mixing (mixer: “Philips) high-protein wheat flour, rice bran flour, granulated sugar, instant
82 dry yeast, bread improver, instant full cream milk powder and bee pollen for 1 minute. After that, water
83 was added and mixing process continued until 20 minutes. Margarine and table salt then added and
84 mixing process continued until minutes. The dough was rested at 26°C for 30 minutes then shaped into a
85 loaf and proofed at 26°C for 90 minutes. After that, it was baked using oven (Maksindo RFL-12C) at 180°C
86 for 30 minutes then cooled at 26°C for 60 minutes (Koeswanto, 2019). Table 1 shows the composition for
87 making bread incorporated with *Monascus*-fermented durian seeds and rice bran and indicate the
88 incorporation of bee pollen at levels control (B₀), 0.075% (B₁), 0.150% (B₂), 0.225% (B₃), 0.300% (B₄) and
89 0.375% (B₅)

90 2.3 *Monascus*-fermented durian seeds preparation

91 Durian seeds were sorted and then washed with water. After that, the durian seeds were boiled
92 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
93 (w/v). The durian seeds were removed from the Ca(OH)₂ solution and then were washed with water. The
94 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
95 were scaled into 50 grams. After that, durian seeds were sterilized at 121°C, 15 lbs/inch² for 10 minutes
96 and were cooled down at 26°C for 30 minutes. The sterilized durian seeds then inoculated with *Monascus*
97 *purpureus* M9 starter and were put into fermentation at 30±1°C for 14 days. The result of the
98 fermentation were dried at 45°C for 24 hour. The dried *Monascus*-fermented durian seed was grinded
99 and sifted to get *Monascus*-fermented durian seeds powder (Puspitadewi *et al.*, 2016).

100 2.4 Sensory evaluation

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

110 2.5 Moisture content

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

114
$$\text{Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)} - \text{weight loss (g)}} \times 100\%$$

115 **2.6 Color test**

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

120 **2.7 Statistical analysis**

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

127 **3. Results and discussion**

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

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

134 Bee pollen contains β -carotene which produce yellow pigment. Bee pollen also contains protein
135 (22.7%); fructose and glucose (25.7%) and sucrose (3.7%) (Komosinska—Vassev *et al.*, 2015). The sugar in

136 bee pollen carried free carbonyl groups and the protein carried free amine groups which then underwent
137 Maillard reaction when exposed to high temperature during baking process and resulted in brown
138 pigment called melanoidin. Higher bee pollen concentration resulted in more yellow and darker crumb.
139 This statement is supported by data collected from color analysis with color reader which is showed in
140 Table 3.

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

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

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

159 influenced by moisture content. Data of moisture content of bread incorporated with *Monascus*-
160 fermented durian seeds and rice bran with each bee pollen concentration is shown in Table 3.

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

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

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

178 This research, however, is subject to limitation. Textural properties was not included in the
179 sensorial evaluation since this research was more focused on improving the taste and aroma of the bread
180 incorporated with *Monascus*-fermented durian seeds and rice bran. This research showed that bread with
181 0.375% had the highest preference score of aroma and taste. Hence, it can be suggested that research

182 regarding textural improvement of bread incorporated with *Monascus*-fermented durian seeds and rice
183 bran with 0.375% bee pollen to be conducted in the future. It's suggested to add hydrocolloid to improve
184 this bread in terms of textural properties by adding hydrocolloid. According to Bourekoua *et al.* (2018)
185 addition of starch or hydrocolloid can improve the textural quality of gluten-free bread.

186 **4. Conclusion**

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

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

197 **Conflict of interest**

198 The authors declare no conflict of interest.

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274

275 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

276

277

278 Table 2. Effect of bee pollen concentration on preference score for bread incorporated with *Monascus*-
 279 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

280 Values are presented as mean±SD (n = 50 for each group). Values with the same superscript within
 281 column are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B:
 282 0% bee pollen, B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen,
 283 and B₅: 0.375% bee pollen

284 Table 3. Effect of bee pollen concentration on color bread incorporated with *Monascus*-fermented durian
 285 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

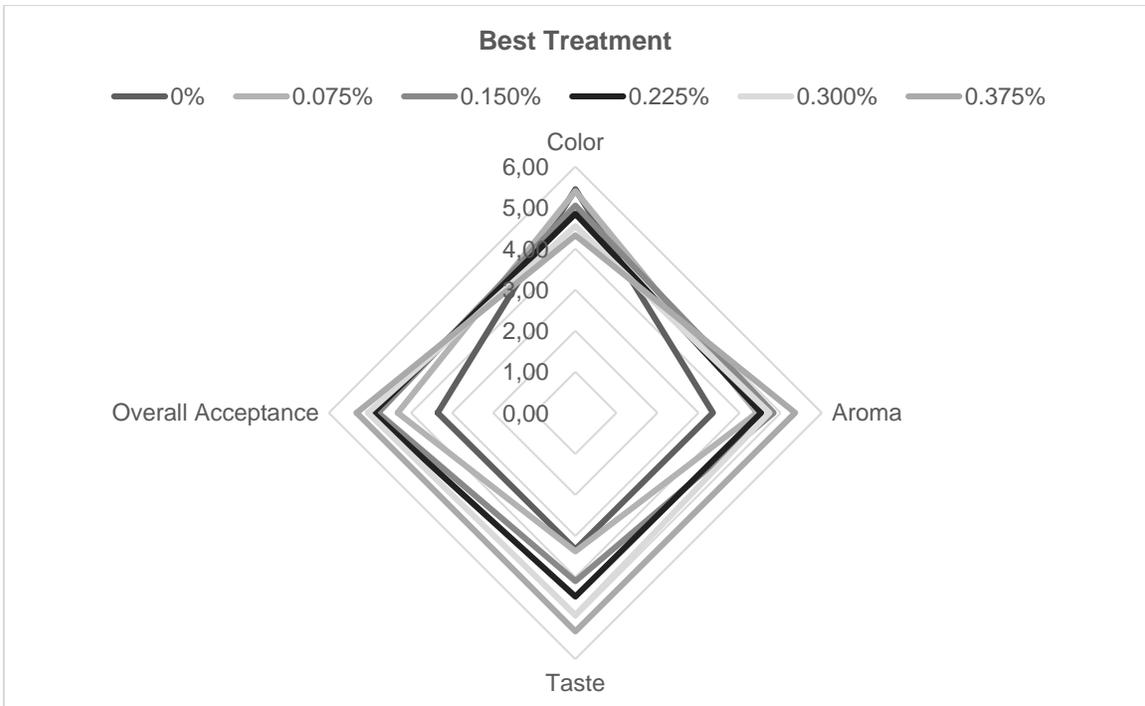
286 Values are presented as mean±SD (n = 4 for each group). Values with the same superscript within column
 287 are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B: 0% bee
 288 pollen, B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and
 289 B₅: 0.375% bee pollen

290

291 Table 4. Effect of bee pollen concentration on the moisture content of bread incorporated with *Monascus*-
292 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

293 Values are presented as mean±SD (n = 4 for each group). Values with the same superscript within column
294 are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$)
295



296

297

Figure 1. Spider web test result

298

2.

Bukti konfirmasi review dan hasil review dari 2 Reviewer

(17 April 2022)

Manuscript ID: FR-2022-036 - ch: x

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Dear Chatarina Yayuk Trisnawati,

Manuscript FR-IFC-036 entitled "Improving the sensory properties of bread incorporated with Monascus-fermented durian seeds and rice bran" which you submitted to Food Research, has been reviewed. The comments of the reviewer(s) are included in the attached file.

The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.

Because we are trying to facilitate timely publication of manuscripts submitted to Food Research, your revised manuscript should be submitted before or by 15th May 2022. If it is not possible for you to submit your revision by this date, please let us know.

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

2 **rice bran**

Commented [A1]: Accommodate the treatment (bee pollen concentrations)

3
4 **Abstract**

5 Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food
6 which contains bioactive compounds. MFDS contains monacolin K which is able reduce cholesterol while
7 RB contains non-dietary fiber, oryzanol, and tocotrienol which can prevent hyperglycemic. Although it is
8 beneficial for human health, it has bitterness and unpleasant aroma that caused by phenolic compounds
9 in MFDS and fatty acids in RB. Incorporation of bee pollen in this bread is one way to improve the sensory
10 properties of bread since it contains volatile compounds with pleasant taste and aroma such as furan,
11 furfural, and pyrazine. The aim of this study was to observe the effect of different bee pollen
12 concentrations on the sensory properties of bread incorporated with MFDS and RB. This study used
13 Randomized Block Design with six levels of treatment starting from 0%; 0.075%; 0.150%; 0.225%; 0.300%;
14 and 0.375%. Data were analyzed by Analysis of Variance with $\alpha = 5\%$. The result of this research showed
15 that different concentration of bee pollen significantly affected the sensory properties of bread
16 incorporated with MFDS and RB. The preference score for color ranged from 4.33 to 5.45; aroma ranged
17 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
18 bee pollen concentration increased, preference score for aroma, taste, and overall acceptance increased
19 and preference score for color decreased. The best treatment was obtained by using 0,375% bee pollen.

Commented [A2]: 0, 0.075....%)

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

21

22 **1. Introduction**

Commented [A3]: Please be focused on the topic, some of the paragraphs are too far from what the study focused on

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

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

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

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

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

Commented [A4]: The title should accommodate this aim

58 2. Materials and methods

59 2.1 Materials

60 Materials that used for making bread incorporated with *Monascus*-fermented durian seeds and
61 rice bran were “Cakra Kembar” high-protein wheat flour, “dr.Liem” rice bran flour, “Fermipan” instant dry
62 yeast, “Dancow” instant full cream milk powder, “Gulaku” granulated sugar, “Bakerine” bread improver,
63 “Cap Kapal” table salt, “Aqua” mineral water, “Blueband” margarine which were purchased from local
64 distributor, “Mirah Delima” multiflora bee pollen which was purchased from Mirah Delima Bee Farm and
65 also *Monascus*-fermented durian seeds powder which was produced in Laboratory of Food Industrial
66 Microbiology, Widya Mandala Catholic University Surabaya. Materials that used for making *Monascus*-
67 fermented durian seeds were Petruk durian seeds, pure culture of *Monascus purpureus* M9, Ca(OH)₂,
68 aquadest and potato dextrose agar (Merck 1.10130.0500).

Commented [A5]: Distilled water

69 2.2 Preparation of bread incorporated with *Monascus*-fermented durian seeds and rice bran

70 Process of making bread incorporated with *Monascus*-fermented durian seeds and rice bran
71 consisted of mixing (mixer: "Philips) high-protein wheat flour, rice bran flour, granulated sugar, instant
72 dry yeast, bread improver, instant full cream milk powder and bee pollen for 1 minute. After that, water
73 was added and mixing process continued until 20 minutes. Margarine and table salt then added and
74 mixing process continued until minutes. The dough was rested at 26°C for 30 minutes then shaped into a
75 loaf and proofed at 26°C for 90 minutes. After that, it was baked using oven (Maksindo RFL-12C) at 180°C
76 for 30 minutes then cooled at 26°C for 60 minutes (Koeswanto, 2019). Table 1 shows the composition for
77 making bread incorporated with *Monascus*-fermented durian seeds and rice bran and indicate the
78 incorporation of bee pollen at levels control (B₀), 0.075% (B₁), 0.150% (B₂), 0.225% (B₃), 0.300% (B₄) and
79 0.375% (B₅)

80 2.3 *Monascus*-fermented durian seeds preparation

81 Durian seeds were sorted and then washed with water. After that, the durian seeds were boiled
82 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
83 (w/v). The durian seeds were removed from the Ca(OH)₂ solution and then were washed with water. The
84 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
85 were scaled into 50 grams. After that, durian seeds were sterilized at 121°C, 15 lbs/inch² for 10 minutes
86 and were cooled down at 26°C for 30 minutes. The sterilized durian seeds then inoculated with *Monascus*
87 *purpureus* M9 starter and were put into fermentation at 30±1°C for 14 days. The result of the
88 fermentation were dried at 45°C for 24 hour. The dried *Monascus*-fermented durian seed was grinded
89 and sifted to get *Monascus*-fermented durian seeds powder (Puspitadewi *et al.*, 2016).

90 2.4 Sensory evaluation

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

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

Commented [A7]: You may add the formulation (the portion of each ingredient for reproducible reason)

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

Commented [A9]: Concentration?

Commented [A10]: Aerobic or anaerobic?

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

100 2.5 Moisture content

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

$$104 \text{ Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)} - \text{weight loss (g)}} \times 100\%$$

105 2.6 Color test

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

110 2.7 Statistical analysis

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

Commented [A12]: Year?

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

117 3. Results and discussion

118 Sensory evaluation were carried using hedonic test method by involving 50 untrained panelist.

119 The result of the test is showed in Table 2.

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

124 Bee pollen contains β -carotene which produce yellow pigment. Bee pollen also contains protein
125 (22.7%); fructose and glucose (25.7%) and sucrose (3.7%) (Komosinska—Vassev *et al.*, 2015). The sugar in
126 bee pollen carried free carbonyl groups and the protein carried free amine groups which then underwent
127 Maillard reaction when exposed to high temperature during baking process and resulted in brown
128 pigment called melanoidin. Higher bee pollen concentration resulted in more yellow and darker crumb.
129 This statement is supported by data collected from color analysis with color reader which is showed in
130 Table 3.

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

136 As seen in Table 2, increased in bee pollen concentration also increased the panelist preference
137 in terms of aroma. Bee pollen consisted of several volatile compounds which comprised of hydrocarbon,

Commented [A13]: 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

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

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

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

159 Bread incorporated with *Monascus*-fermented durian seeds and rice bran achieved higher overall
160 acceptance score as the bee pollen concentration increased as seen in Table 2. This result showed that

Commented [A14]: The astringent taste of fermented durian seed has never been mentioned before. You may put the information of the four characteristics in the introduction

Commented [A15]: What are they? and how many? (are they dominant?)

161 panelist preferred bread incorporated with *Monascus*-fermented durian seeds and rice bran with higher
162 bee pollen concentration since the taste and aroma was better, even though the color was less favorable.

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

168 This research, however, is subject to limitation. Textural properties was not included in the
169 sensorial evaluation since this research was more focused on improving the taste and aroma of the bread
170 incorporated with *Monascus*-fermented durian seeds and rice bran. This research showed that bread with
171 0.375% had the highest preference score of aroma and taste. Hence, it can be suggested that research
172 regarding textural improvement of bread incorporated with *Monascus*-fermented durian seeds and rice
173 bran with 0.375% bee pollen to be conducted in the future. It's suggested to add hydrocolloid to improve
174 this bread in terms of textural properties by adding hydrocolloid. According to Bourekoua *et al.* (2018)
175 addition of starch or hydrocolloid can improve the textural quality of gluten-free bread.

176 4. Conclusion

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

182 This research provided new insight into the field of bakery products which are supplemented with
183 ingredients derived from food waste such as rice bran and durian seeds. Through this research, it can be

Commented [A16]: ? why

184 found out that addition of bee pollen helps to improve the taste and aroma of bread with food waste
185 derived ingredients. Researcher may also consider addition of bee pollen to improve the taste and aroma
186 of other bakery products which contains food waste derived ingredients.

187 **Conflict of interest**

188 The authors declare no conflict of interest.

189 **Acknowledgments**

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191 Indonesia through competitive research “Penelitian Terapan Unggulan Perguruan Tinggi (PTUPT)” with
192 contract number 130X/WM01.5/N/2020.

193

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265

266 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

Commented [A18]: Where is the durian seeds flour?
And what is B -B5? Add description

267

268

269 Table 2. Effect of bee pollen concentration on preference score for bread incorporated with *Monascus*-
 270 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

271 Values are presented as mean±SD (n = 50 for each group). Values with the same superscript within
 272 column are not significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B:
 273 0% bee pollen, B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen,
 274 and B₅: 0.375% bee pollen

275 Table 3. Effect of bee pollen concentration on color bread incorporated with *Monascus*-fermented durian
 276 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

277 Values are presented as mean±SD (n = 4 for each group). Values with the same superscript within column

278 are not significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B: 0% bee

279 pollen, B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and

280 B₅: 0.375% bee pollen

281

282 Table 4. Effect of bee pollen concentration on the moisture content of bread incorporated with *Monascus*-
283 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

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

286

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

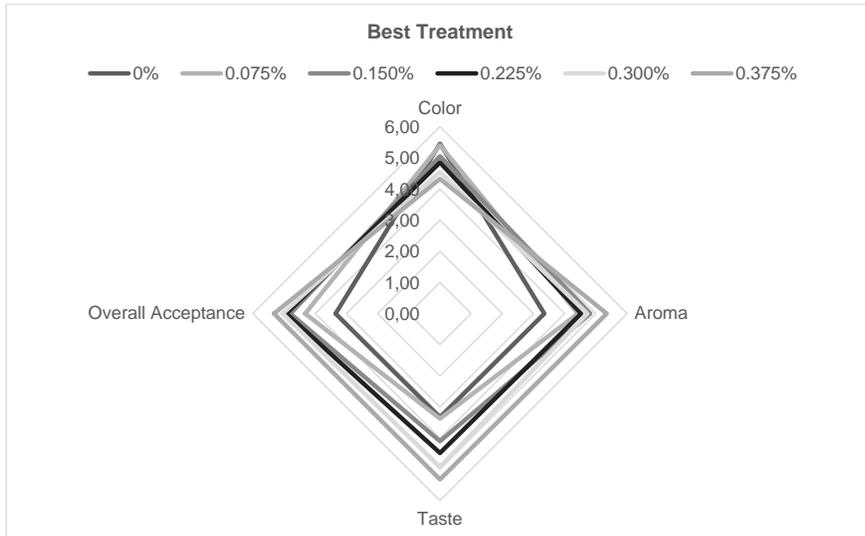


Figure 1. Spider web test result

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Date : 11th March 2022

Manuscript ID : FR-IFC-036

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

1. IF YOU CANNOT REVIEW THIS MANUSCRIPT OR MEET THE DEADLINE, PLEASE INFORM US WITHOUT DELAY.
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Using item 2 in page 1 as a guideline, please indicate the reasons for your recommendations. Most author(s) will appreciate frankness, combined with a modicum of tact. Even if you recommend that the manuscript be accepted for publication, please provide some general comments to the author(s).

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	✓				

<p>(REVIEWER'S SECTION)</p> <p>REVIEWER'S COMMENTS/SUGGESTIONS</p>		<p>(AUTHOR'S SECTION)</p> <p>AUTHOR'S ACTION/RESPONSE</p>
		<p>*NOTE FOR AUTHOR: Please state your response to the reviewer's comments/suggestion below</p>
<p>1.</p>	<p>Title <i>It should reflect the article</i> 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.</p>	
<p>2.</p>	<p>Abstract <i>Background, Aim, Methodology and Conclusion</i> Still not shown which part was improved OK</p>	
<p>3.</p>	<p>Keywords <i>Min. 3 and Max. 6</i> OK</p>	
<p>4.</p>	<p>Introduction <i>Concise with sufficient background</i> OK</p>	
<p>5.</p>	<p>Research design/Methodology <i>Clearly described and reproducible</i></p> <ul style="list-style-type: none"> • Material: is it necessary to write the commercial brand? (line 61-53) • Not consistent in writing the symbol of the treatments (line 78-79) 	
<p>6.</p>	<p>Data Analysis <i>Results well presented and discussed</i></p> <ul style="list-style-type: none"> • Not consistent used of "$p \leq 0,05$". 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 	

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	<p>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)?</p> <ul style="list-style-type: none"> • 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 <i>Monascus</i>-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.	<p>Conclusion <i>A clear summary of the study</i></p> <ul style="list-style-type: none"> • 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.	<p>References <i>References should follow the journal's format</i></p> <p>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.</p>	
9.	<p>English Proficiency OK</p>	
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As Figure 1 is shown not in color, please make the gradation grayscale color which is easy to understand.	
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Overall Evaluation

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Evaluation Criteria	Grade				
	A (Excellent)	B	C	D	E (Worst)
1. Appropriateness of Contents				x	
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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(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
1.	Title <i>It should reflect the article</i> The title has not reflected the content of the article. Please accomodate the treatment	
2.	Abstract <i>Background, Aim, Methodology and Conclusion</i> Abstract is fine	
3.	Keywords <i>Min. 3 and Max. 6</i> The keywords is fine	
4.	Introduction <i>Concise with sufficient background</i> 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 <i>Clearly described and reproducible</i> 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 <i>Results well presented and discussed</i> 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	

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	<i>A clear summary of the study</i> Conclusion is fine	
8.	References <i>References should follow the journal's format</i> Please use more recent and related references	
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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	

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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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<p>4.</p>	<p>Introduction <i>Concise with sufficient background</i></p> <p>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</p>	<p>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.</p>
<p>5.</p>	<p>Research design/Methodology <i>Clearly described and reproducible</i></p> <p>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</p>	<p>I've included the MFDS flour in the formulation table and added another parameter</p>
<p>6.</p>	<p>Data Analysis <i>Results well presented and discussed</i></p> <p>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</p>	<p>I've put the effect of incorporating not only bee pollen but also rice bran and MFDS on the physicochemical properties of the bread.</p>

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	and the possible interaction among compounds	
7.	Conclusion <i>A clear summary of the study</i> Conclusion is fine	
8.	References <i>References should follow the journal's format</i> 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	I've put more analyzed parameters in the paper and written the discussion for each parameter.

Overall Evaluation

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<p>4.</p>	<p>Introduction <i>Concise with sufficient background</i> OK</p>	
<p>5.</p>	<p>Research design/Methodology <i>Clearly described and reproducible</i></p> <ul style="list-style-type: none"> • Material: is it necessary to write the commercial brand? (line 61-53) • Not consistent in writing the symbol of the treatments (line 78-79) 	<p>I've included the MFDS flour in the formulation table and added another parameter</p>
<p>6.</p>	<p>Data Analysis <i>Results well presented and discussed</i></p> <ul style="list-style-type: none"> • Not consistent used of "$p > 0,05$". Some part still using \leq (line 4, 125, 272, 278, 285) 	<p>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.</p>

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	<ul style="list-style-type: none"> • 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 <i>Monascus</i>-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.	<p>References</p>	

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	<i>References should follow the journal's format</i> 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.

Overall Evaluation

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

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4
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14

15 **Abstract**

16 Bread incorporated with *Monascus*-fermented durian seeds (MFDS) and rice bran (RB) is a functional food
17 which contains bioactive compounds. Although it is beneficial for human health, it has bitterness and
18 unpleasant aroma that caused by phenolic compounds in MFDS and fatty acids in RB. Incorporation of
19 bee pollen in this bread is one way to improve the sensory properties of bread. The aim of this study was
20 to observe the effect of different bee pollen concentrations on the physicochemical and sensory
21 properties of bread incorporated with MFDS and RB. This study used Randomized Block Design with six
22 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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23 of Variance with $\alpha = 5\%$. The results indicated that difference in bee pollen concentration significantly
24 affected on physicochemical and sensory properties of bread incorporated with MFDS and RB ($p>0.05$).
25 Higher bee pollen concentration significantly increased the preference score for taste, aroma and overall
26 acceptance ($p>0.05$). The best treatment was obtained by using 0.375% bee pollen.

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

28 1. Introduction

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

36 Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and
37 taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains
38 monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty
39 acids which have rancid properties (Cho and Samuel, 2009). MFDS contains secondary metabolite such as
40 tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.*,
41 2016; Hasim *et al.*, 2019).

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

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

49 2. Materials and methods

50 2.1 Materials

51 Materials that used for making bread incorporated with MFDS and RB were bread flour, rice bran
52 flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table salt,
53 mineral water, margarine which were purchased from local distributor, "Mirah Delima" multiflora bee
54 pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which was produced
55 in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University Surabaya. Materials
56 that used for producing MFDS flour were Petruk durian seeds, pure culture of *Monascus purpureus*
57 M9, Ca(OH)₂, distilled water and potato dextrose agar (Merck 1.10130.0500).

58 59 2.2 Preparation of bread incorporated with *Monascus-fermented durian seeds* and rice bran

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

67 2.3 *Monascus-fermented durian seeds* preparation

68 Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that,
69 the durian seeds were boiled with 5% Ca(OH)₂ solution at 85-90°C for 10 minutes [durian seeds: 5%

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Commented [A4R3]:

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70 Ca(OH)₂ solution = 1:1 (w/v)]. The durian seeds were removed from the Ca(OH)₂ solution and then
71 washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through
72 first drying process at 45°C for 40 minutes. The durian seeds were then weighed into 50 g. After that,
73 durian seeds were sterilized at 121°C, 15 lbs/inch² for 10 minutes and cooled down at 26°C for 30
74 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w)
75 and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were
76 dried at 45°C for 24 hours, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi
77 *et al.*, 2016).

Commented [A6]: Concentration?

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78 2.3 Moisture content analysis

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

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$$81 \text{ Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)} - \text{weight loss (g)}} \times 100\%$$

82 2.4 Specific volume

83 Specific volume determination was carried out using seed displacement method according to
84 Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds
85 which were above the container rim were then removed using straight ruler. All the foxtail millet
86 seeds in the container were then poured into a measuring cylinder to measure the volume of the
87 container (V₁). This steps were then repeated except that a loaf of bread sample was already inside
88 the container before it was filled with seeds to obtain V₂.

$$89 \text{ Specific volume (cm}^3/\text{g)} = \frac{V_1 \text{ (ml)} - V_2 \text{ (ml)}}{W \text{ (g)}}$$

90 2.5 Texture analysis

91 Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out
92 using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential

93 compression events (probe: P36/R, pre-test speed: 5 mm s⁻¹, test speed: 1.5 mm s⁻¹, post-test speed
94 10 mm s⁻¹, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

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

99 2.6 Color analysis

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

103 2.8 Sensory evaluation

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

110 2.9 Statistical analysis

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

114 3. Results and discussion

115 3.1 Moisture Content

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

116 According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture
117 content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that
118 maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice
119 bran contains 7-11% fiber (Henderson *et al.*, 2012). Ability of fiber to bind with water then resulted
120 in increased moisture content of bread (Sangle *et al.*, 2017). Bee pollen contains glucose, fructose and
121 sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*,
122 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were
123 hydrophilic amino acid. (Taha *et al.*, 2017). Conte *et al.* (2020) also reported that moisture content of
124 gluten free bread increased as bee pollen concentration increased.

125 3.2 Physical properties

126 Increase of specific volume in bread samples with 0-0.15% bee pollen was due to additional sugar
127 from bee pollen which are useful CO₂ production by yeast. The CO₂ pushed the gluten matrix to
128 become thinner and resulted in reduced hardness. More CO₂ gas also increased viscoelasticity of
129 gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a
130 decrease in specific volume due to competition in binding water between the hydrophilic molecules
131 (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker
132 and could not retain CO₂ as much as bread with 0-0.15%. Lower retention of CO₂ also resulted in bread
133 with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease
134 bread's springiness.

135 The results in Table 5. showed that as bee pollen concentration increased, L* value decreased and
136 a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of
137 rice bran flour since it is brown in color and Maillard reaction which occurred during baking process.
138 Starowicz and Zieliński (2019) reported that Free amine groups in protein and carbonyl groups in
139 sugars went through Maillard reaction to produce melanoidin which reduce L*value of food Brown's

140 base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value
141 of b* was also influenced by the yellow pigment β -carotene in bee pollen.

142 3.3 Sensory evaluation

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

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

154 4. Conclusion

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

160 Conflict of interest

161 The authors declare no conflict of interest.

162 Acknowledgments

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165 contract number 130X/WM01.5/N/2020.

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236

237 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

Commented [A12]: Where is the durian seeds flour?
And what is B -B5? Add description

238 B₀ : 0% bee pollen; B₁ : 0.075% bee pollen; B₂ : 0.150% bee pollen; B₃ : 0.225% bee pollen; B₄ : 0.300% bee
239 pollen; B₅ : 0.375% bee pollen

240

241 Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented
 242 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

243 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 244 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 245 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 246 bee pollen

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

247
 248 Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented
 249 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

250 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 251 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 252 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 253 bee pollen

254
 255
 256

257 Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented
 258 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

259 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 260 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 261 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 262 bee pollen

263

264

265 Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and
 266 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

267 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 268 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 269 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 270 bee pollen

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

273 Values are presented as mean±SD (n = 50). Values with the same superscript within column are not
 274 significantly ($p > 0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 275 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 276 bee pollen

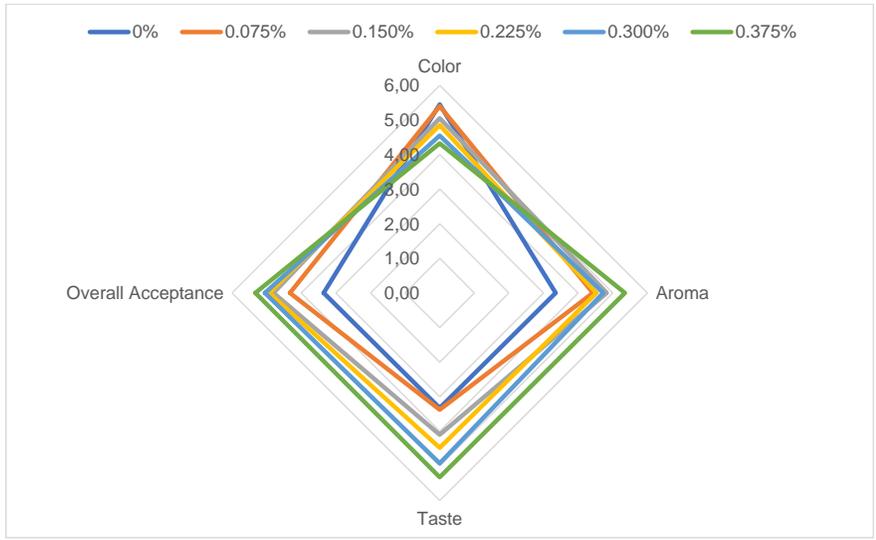


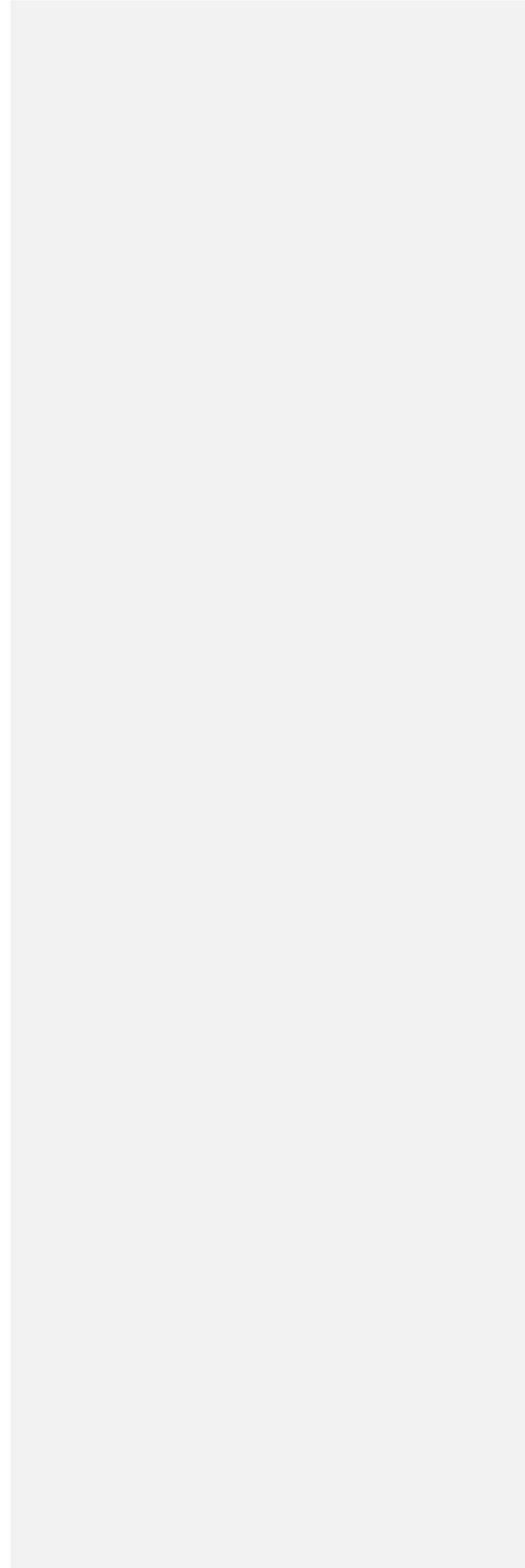
Figure 1. Spider web test result

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

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

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14

15 **Abstract**

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

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

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

28 1. Introduction

29 The development of functional bakery products had been widely studied among food scientists
30 as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.*
31 (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented
32 durian seeds (MFDS) into bread. Rice bran contains γ -orizanol, γ -tocotrienol and non-dietary fibre which
33 can regulate blood sugar levels (Premakumari *et al.*, 2013; Sivamaruthi *et al.*, 2018). MFDS contains
34 monascin pigments that can help to reduce blood sugar level and monacolin-K which helps to reduce
35 cholesterol production. (Nugerahani *et al.*, 2017; Faroukh and Baumgärtel, 2019).

36 Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and
37 taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains
38 monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty
39 acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as
40 tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.*,
41 2016; Hasim *et al.*, 2019).

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

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

50 **2. Materials and methods**

51 *2.1 Materials*

52 Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice
53 bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table
54 salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima"
55 multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which
56 was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University
57 Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture
58 of *Monascus purpureus* M9, Ca(OH)₂, distilled water and potato dextrose agar (Merck 1.10130.0500).

59

60 *2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran*

61 Sample preparation began by mixing all of the dry ingredients except table salt for 1 minute and
62 followed by adding water. The mixing process then continued for 10 minutes. Margarine and table
63 salt was then added and the mixing process continued for 5 minutes. The dough was fermented at
64 26°C for 30 minutes. After the dough was shaped into a loaf the dough was proofed at 26°C for 90
65 minutes. The bread was baked with the oven at 180°C for 30 minutes then cooled at 26°C for 60
66 minutes. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels
67 of bee pollen concentration.

68 *2.3 Monascus-fermented durian seeds preparation*

69 Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that,
70 the durian seeds were boiled with 5% Ca(OH)₂ solution at 85-90°C for 10 minutes [durian seeds: 5%
71 Ca(OH)₂ solution = 1:1 (w/v)]. The durian seeds were removed from the Ca(OH)₂ solution and then
72 washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through
73 first drying process at 45°C for 40 minutes. The durian seeds were then weighed into 50 g. After that,
74 durian seeds were sterilized at 121°C, 15 lbs/inch² for 10 minutes and cooled down at 26°C for 30
75 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w)
76 and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were
77 dried at 45°C for 24 hours, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi
78 *et al.*, 2016).

79 2.3 Moisture content analysis

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

$$82 \text{ Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)} - \text{weight loss (g)}} \times 100\%$$

83 2.4 Specific volume

84 Specific volume determination was carried out using seed displacement method according to
85 Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds
86 which were above the container rim were then removed using straight ruler. All the foxtail millet
87 seeds in the container were then poured into a measuring cylinder to measure the volume of the
88 container (V₁). This steps were then repeated except that a loaf of bread sample was already inside
89 the container before it was filled with seeds to obtain V₂.

$$90 \text{ Specific volume (cm}^3/\text{g)} = \frac{V_1 \text{ (ml)} - V_2 \text{ (ml)}}{W \text{ (g)}}$$

91 2.5 Texture analysis

92 Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out
93 using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential
94 compression events (probe: P36/R, pre-test speed: 5 mm s⁻¹, test speed: 1.5 mm s⁻¹, post-test speed
95 10 mm s⁻¹, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

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

100 2.6 Color analysis

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

104 2.8 Sensory evaluation

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

111 2.9 Statistical analysis

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

115 3. Results and discussion

116 3.1 *Moisture Content*

117 According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture
118 content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that
119 maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice
120 bran contains 7-11% fiber (Henderson *et al.*, 2012). Ability of fiber to bind with water then resulted
121 in increased moisture content of bread (Sangle *et al.*, 2017). Bee pollen contains glucose, fructose and
122 sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*,
123 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were
124 hydrophilic amino acid. (Taha *et al.*, 2017). Conte *et al.* (2020) also reported that moisture content of
125 gluten free bread increased as bee pollen concentration increased.

126 3.2 *Physical properties*

127 Increase of specific volume in bread samples with 0-0.15% bee pollen was due to additional sugar
128 from bee pollen which are useful CO₂ production by yeast. The CO₂ pushed the gluten matrix to
129 become thinner and resulted in reduced hardness. More CO₂ gas also increased viscoelasticity of
130 gluten matrix hence springiness increased. Bread samples with 0.225-0.375% bee pollen showed a
131 decrease in specific volume due to competition in binding water between the hydrophilic molecules
132 (protein and sugar) in bee pollen and gluten forming protein. Hence the gluten matrix became weaker
133 and could not retain CO₂ as much as bread with 0-0.15%. Lower retention of CO₂ also resulted in bread
134 with thicker gluten matrix and lower viscoelasticity hence increase bread hardness and decrease
135 bread's springiness.

136 The results in Table 5 showed that as bee pollen concentration increased, L* value decreased and
137 a*, b*, C and H value of bread samples increased. Lower L* might be attributed to incorporation of
138 rice bran flour since it is brown in color and Maillard reaction which occurred during baking process.
139 Starowicz and Zieliński (2019) reported that Free amine groups in protein and carbonyl groups in

140 sugars went through Maillard reaction to produce melanoidin which reduce L*value of food Brown's
141 base colors are grounded with red and yellow which resulted in increased of a* and b* value. Value
142 of b* was also influenced by the yellow pigment β -carotene in bee pollen.

143 3.3 Sensory evaluation

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

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

155 4. Conclusion

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

161 Conflict of interest

162 The authors declare no conflict of interest.

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235 van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology*
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237

238 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

239 B₀ : 0% bee pollen; B₁ : 0.075% bee pollen; B₂ : 0.150% bee pollen; B₃ : 0.225% bee pollen; B₄ : 0.300% bee
 240 pollen; B₅ : 0.375% bee pollen

241

242 Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented
243 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

244 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
245 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
246 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
247 bee pollen

248
249 Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented
250 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

251 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
252 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
253 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
254 bee pollen

255
256
257

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258 Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented
 259 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

260 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 261 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 262 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 263 bee pollen

264
 265
 266 Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and
 267 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

268 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 269 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 270 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 271 bee pollen

272 Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian
 273 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±SD (n = 50). Values with the same superscript within column are not
 275 significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 276 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 277 bee pollen

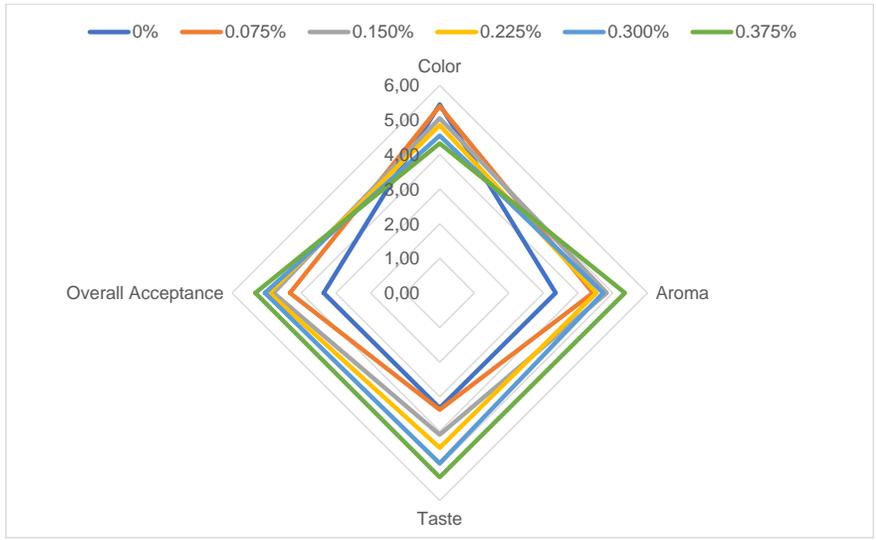


Figure 1. Spider web test result

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

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14

15 **Abstract**

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

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

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

28 1. Introduction

29 The development of functional bakery products had been widely studied among food scientists
30 as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.*
31 (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented
32 durian seeds (MFDS) into bread. Rice bran contains γ -orizanol, γ -tocotrienol and fibre which can regulate
33 blood sugar levels (Premakumari *et al.*, 2013; Sivamaruthi *et al.*, 2018). MFDS contains monascin pigments
34 that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production.
35 (Nugerahani *et al.*, 2017; Faroukh and Baumgärtel, 2019).

36 Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and
37 taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains
38 monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty
39 acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as
40 tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.*,
41 2016; Hasim *et al.*, 2019).

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

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

50 **2. Materials and methods**

51 *2.1 Materials*

52 Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice
53 bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table
54 salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima"
55 multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which
56 was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University
57 Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture
58 of *Monascus purpureus* M9, Ca(OH)₂, distilled water and potato dextrose agar (Merck 1.10130.0500).

59 *2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran*

60 Sample preparation began by mixing all of the dry ingredients except table salt for 1 minute and
61 followed by adding water. The mixing process then continued for 10 minutes. Margarine and table
62 salt was then added and the mixing process continued for 5 minutes. The dough was fermented at
63 26°C for 30 minutes. After the dough was shaped into a loaf the dough was proofed at 26°C for 90
64 minutes. The bread was baked with the oven at 180°C for 30 minutes then cooled at 26°C for 60
65 minutes. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels
66 of bee pollen concentration.

67 *2.3 Monascus-fermented durian seeds preparation*

68 Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that,
69 the durian seeds were boiled with 5% Ca(OH)₂ solution at 85-90°C for 10 minutes [durian seeds: 5%

70 Ca(OH)₂ solution = 1:1 (w/v)]. The durian seeds were removed from the Ca(OH)₂ solution and then
71 washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through
72 first drying process at 45°C for 40 minutes. The durian seeds were then weighed into 50 g. After that,
73 durian seeds were sterilized at 121°C, 15 lbs/inch² for 10 minutes and cooled down at 26°C for 30
74 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w)
75 and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were
76 dried at 45°C for 24 hours, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi
77 *et al.*, 2016).

78 2.3 Moisture content analysis

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

$$82 \text{ Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)} - \text{weight loss (g)}} \times 100\%$$

83 2.4 Specific volume

84 Specific volume determination was carried out using seed displacement method according to
85 Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds
86 which were above the container rim were then removed using straight ruler. All the foxtail millet
87 seeds in the container were then poured into a measuring cylinder to measure the volume of the
88 container (V₁). These steps were then repeated except that a loaf of bread sample was already inside
89 the container before it was filled with seeds to obtain V₂.

$$90 \text{ Specific volume (cm}^3/\text{g)} = \frac{V_1 \text{ (ml)} - V_2 \text{ (ml)}}{W \text{ (g)}}$$

91

92

93 *2.5 Texture analysis*

94 Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out
95 using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential
96 compression events (probe: P36/R, pre-test speed: 5 mm s⁻¹, test speed: 1.5 mm s⁻¹, post-test speed
97 10 mm s⁻¹, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

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

102 *2.6 Color analysis*

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

106 *2.8 Sensory evaluation*

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

113 *2.9 Statistical analysis*

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

117 3. Results and discussion

118 3.1 Moisture Content

119 According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture
120 content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that
121 maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice
122 bran contains 7-11% fiber (Henderson *et al.*, 2012). Ability of fiber to bind with water then resulted
123 in increased moisture content of bread (Sangle *et al.*, 2017). Bee pollen contains glucose, fructose and
124 sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*,
125 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were
126 hydrophilic amino acid. (Taha *et al.*, 2017). Conte *et al.* (2020) also reported that moisture content of
127 gluten free bread increased as bee pollen concentration increased.

128 3.2 Physical properties

129 Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due
130 to additional sugar from bee pollen which are useful CO₂ production by yeast. The CO₂ pushed the
131 gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO₂ gas
132 also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-
133 0.375% bee pollen showed a decrease in specific volume due to competition in binding water between
134 the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the
135 gluten matrix became weaker and could not retain CO₂ as much as bread with 0-0.15%. Lower
136 retention of CO₂ also resulted in bread with thicker gluten matrix and lower viscoelasticity hence
137 increase bread hardness and decrease bread's springiness.

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

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

145 3.3 Sensory evaluation

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

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

158 4. Conclusion

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

164

165 **Conflict of interest**

166 The authors declare no conflict of interest.

167 **Acknowledgments**

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169 Indonesia through competitive research “Penelitian Terapan Unggulan Perguruan Tinggi (PTUPT)” with
170 contract number 130X/WM01.5/N/2020.

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241

242 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

243 B₀ : 0% bee pollen; B₁ : 0.075% bee pollen; B₂ : 0.150% bee pollen; B₃ : 0.225% bee pollen; B₄ : 0.300% bee
 244 pollen; B₅ : 0.375% bee pollen

245

246 Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented
247 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

248 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
249 significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
250 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
251 bee pollen

252

253 Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented
254 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

255 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
256 significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
257 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
258 bee pollen

259

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262 Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented
 263 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

264 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 265 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 266 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 267 bee pollen

268

269

270 Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and
 271 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

272 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 273 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 274 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 275 bee pollen

276 Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian
 277 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

278 Values are presented as mean±SD (n = 50). Values with the same superscript within column are not
 279 significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 280 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 281 bee pollen

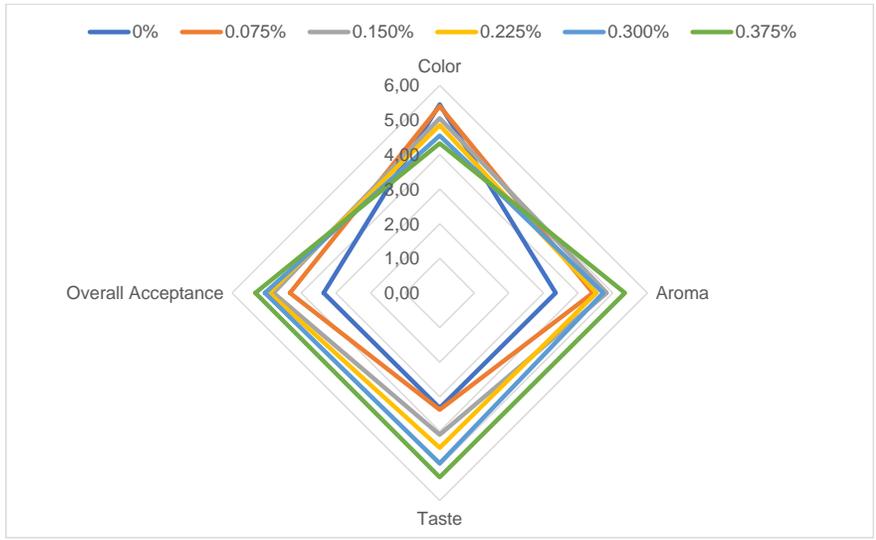


Figure 1. Spider web test result

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

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14

15 **Abstract**

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

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

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

28 1. Introduction

29 The development of functional bakery products had been widely studied among food scientists
30 as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.*
31 (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented
32 durian seeds (MFDS) into bread. Rice bran contains γ -orizanol, γ -tocotrienol and fibre which can regulate
33 blood sugar levels (Premakumari *et al.*, 2013; Sivamaruthi *et al.*, 2018). MFDS contains monascin pigments
34 that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production.
35 (Nugerahani *et al.*, 2017; Faroukh and Baumgärtel, 2019).

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36 Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and
37 taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains
38 monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty
39 acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as
40 tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.*,
41 2016; Hasim *et al.*, 2019).

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

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

50 2. Materials and methods

51 2.1 Materials

52 Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice
53 bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table
54 salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima"
55 multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which
56 was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University
57 Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture
58 of *Monascus purpureus* M9, Ca(OH)₂, distilled water and potato dextrose agar (Merck 1.10130.0500).

59 2.2 Preparation of bread incorporated with *Monascus-fermented durian seeds* and rice bran

60 Sample preparation began by mixing all of the dry ingredients except table salt for 1 minute and
61 followed by adding water. The mixing process then continued for 10 minutes. Margarine and table
62 salt was then added and the mixing process continued for 5 minutes. The dough was fermented at
63 26°C for 30 minutes. After the dough was shaped into a loaf the dough was proofed at 26°C for 90
64 minutes. The bread was baked with the oven at 180°C for 30 minutes then cooled at 26°C for 60
65 minutes. Table 1 shows the composition of bread incorporated with MFDS and RB with different levels
66 of bee pollen concentration.

67 2.3 *Monascus-fermented durian seeds* preparation

68 Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that,
69 the durian seeds were boiled with 5% Ca(OH)₂ solution at 85-90°C for 10 minutes [durian seeds: 5%

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70 Ca(OH)₂ solution = 1:1 (w/v)]. The durian seeds were removed from the Ca(OH)₂ solution and then
71 washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through
72 first drying process at 45°C for 40 minutes. The durian seeds were then weighed into 50 g. After that,
73 durian seeds were sterilized at 121°C, 15 lbs/inch² for 10 minutes and cooled down at 26°C for 30
74 minutes. The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w)
75 and were put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were
76 dried at 45°C for 24 hours, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi
77 *et al.*, 2016).

78 2.3 Moisture content analysis

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

$$82 \text{ Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)} - \text{weight loss (g)}} \times 100\%$$

83 2.4 Specific volume

84 Specific volume determination was carried out using seed displacement method according to
85 Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds
86 which were above the container rim were then removed using straight ruler. All the foxtail millet
87 seeds in the container were then poured into a measuring cylinder to measure the volume of the
88 container (V₁). These steps were then repeated except that a loaf of bread sample was already inside
89 the container before it was filled with seeds to obtain V₂.

$$90 \text{ Specific volume (cm}^3/\text{g)} = \frac{V_1 \text{ (ml)} - V_2 \text{ (ml)}}{W \text{ (g)}}$$

91

92

93 2.5 *Texture analysis*

94 Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out
95 using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential
96 compression events (probe: P36/R, pre-test speed: 5 mm s⁻¹, test speed: 1.5 mm s⁻¹, post-test speed
97 10 mm s⁻¹, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

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

102 2.6 *Color analysis*

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

106 2.8 *Sensory evaluation*

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

113 2.9 *Statistical analysis*

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

117 3. Results and discussion

118 3.1 Moisture Content

119 According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture
120 content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that
121 maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice
122 bran contains 7-11% fiber (Henderson *et al.*, 2012). Ability of fiber to bind with water then resulted
123 in increased moisture content of bread (Sangle *et al.*, 2017). Bee pollen contains glucose, fructose and
124 sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*,
125 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were
126 hydrophilic amino acid- (Taha *et al.*, 2017). Conte *et al.* (2020) also reported that moisture content of
127 gluten free bread increased as bee pollen concentration increased.

128 3.2 Physical properties

129 Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due
130 to additional sugar from bee pollen which are useful CO₂ production by yeast. The CO₂ pushed the
131 gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO₂ gas
132 also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-
133 0.375% bee pollen showed a decrease in specific volume due to competition in binding water between
134 the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the
135 gluten matrix became weaker and could not retain CO₂ as much as bread with 0-0.15%. Lower
136 retention of CO₂ also resulted in bread with thicker gluten matrix and lower viscoelasticity hence
137 increase bread hardness and decrease bread's springiness.

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

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

145 3.3 Sensory evaluation

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

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

158 4. Conclusion

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

164

165 **Conflict of interest**

166 The authors declare no conflict of interest.

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241

242 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

243 B₀ : 0% bee pollen; B₁ : 0.075% bee pollen; B₂ : 0.150% bee pollen; B₃ : 0.225% bee pollen; B₄ : 0.300% bee
 244 pollen; B₅ : 0.375% bee pollen

245

246 Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented
247 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

248 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
249 significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
250 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
251 bee pollen

252

253 Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented
254 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

255 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
256 significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
257 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
258 bee pollen

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260

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262 Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented
 263 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

264 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 265 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 266 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 267 bee pollen

268
 269
 270 Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and
 271 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

272 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 273 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 274 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 275 bee pollen

276 Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian
 277 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

278 Values are presented as mean±SD (n = 50). Values with the same superscript within column are not
 279 significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 280 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 281 bee pollen

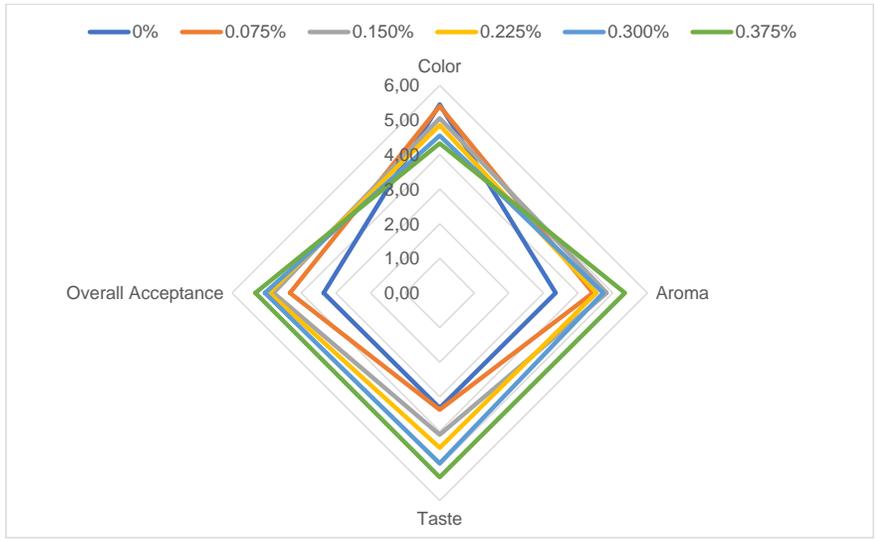


Figure 1. Spider web test result

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

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15 **Abstract**

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

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

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

28 1. Introduction

29 The development of functional bakery products had been widely studied among food scientists
30 as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.*
31 (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented
32 durian seeds (MFDS) into bread. Rice bran contains γ -orizanol, γ -tocotrienol and fibre which can regulate
33 blood sugar levels (Premakumari *et al.*, 2013; Sivamaruthi *et al.*, 2018). MFDS contains monascin pigments
34 that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production.
35 (Nugerahani *et al.*, 2017; Faroukh and Baumgärtel, 2019).

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36 Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and
37 taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains
38 monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty
39 acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as
40 tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.*,
41 2016; Hasim *et al.*, 2019).

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

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

50 2. Materials and methods

51 2.1 Materials

52 Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice
53 bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table
54 salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima"
55 multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which
56 was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University
57 Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture
58 of *Monascus purpureus* M9, Ca(OH)₂, distilled water and potato dextrose agar (Merck 1.10130.0500).

59 2.2 Preparation of bread incorporated with *Monascus-fermented durian seeds* and rice bran

60 Sample preparation began by mixing all of the dry ingredients except table salt for 1 minute and
61 followed by adding water. The mixing process then continued for 10 minutes. Margarine and table salt
62 was then added and the mixing process continued for 5 minutes. The dough was fermented at 26°C for
63 30 minutes. After the dough was shaped into a loaf the dough was proofed at 26°C for 90 minutes. The bread
64 was baked with the oven at 180°C for 30 minutes then cooled at 26°C for 60 minutes. Table 1 shows the
65 composition of bread incorporated with MFDS and RB with different levels of bee pollen
66 concentration.

67 2.3 *Monascus-fermented durian seeds* preparation

68 Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that,
69 the durian seeds were boiled with 5% Ca(OH)₂ solution at 85-90°C for 10 minutes [durian seeds: 5%

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70 Ca(OH)₂ solution = 1:1 (w/v)]. The durian seeds were removed from the Ca(OH)₂ solution and then
71 washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through
72 first drying process at 45°C for 40 minutes. The durian seeds were then weighed into 50 g. After that,
73 durian seeds were sterilized at 121°C, 15 lbs/inch² for 10 minutes and cooled down at 26°C for 30 minutes.
74 The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were
75 put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at
76 45°C for 24 hours, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.*,
77 2016).

78 2.3 Moisture content analysis

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

$$82 \text{ Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)} - \text{weight loss (g)}} \times 100\%$$

83 2.4 Specific volume

84 Specific volume determination was carried out using seed displacement method according to
85 Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds
86 which were above the container rim were then removed using straight ruler. All the foxtail millet
87 seeds in the container were then poured into a measuring cylinder to measure the volume of the
88 container (V₁). These steps were then repeated except that a loaf of bread sample was already inside
89 the container before it was filled with seeds to obtain V₂.

$$90 \text{ Specific volume (cm}^3/\text{g)} = \frac{V_1 \text{ (ml)} - V_2 \text{ (ml)}}{W \text{ (g)}}$$

91

92

93 2.5 *Texture analysis*

94 Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out
95 using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential
96 compression events (probe: P36/R, pre-test speed: 5 mm s⁻¹, test speed: 1.5 mm s⁻¹, post-test speed
97 10 mm s⁻¹, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

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

102 2.6 *Color analysis*

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

106 2.8 *Sensory evaluation*

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

113 2.9 *Statistical analysis*

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

117 3. Results and discussion

118 3.1 Moisture Content

119 According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture
120 content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that
121 maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice
122 bran contains 7-11% fiber (Henderson *et al.*, 2012). Ability of fiber to bind with water then resulted
123 in increased moisture content of bread (Sangle *et al.*, 2017). Bee pollen contains glucose, fructose and
124 sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*,
125 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were
126 hydrophilic amino acid- (Taha *et al.*, 2017). Conte *et al.* (2020) also reported that moisture content of
127 gluten free bread increased as bee pollen concentration increased.

128 3.2 Physical properties

129 Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due
130 to additional sugar from bee pollen which are useful CO₂ production by yeast. The CO₂ pushed the
131 gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO₂ gas
132 also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-
133 0.375% bee pollen showed a decrease in specific volume due to competition in binding water between
134 the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the
135 gluten matrix became weaker and could not retain CO₂ as much as bread with 0-0.15%. Lower
136 retention of CO₂ also resulted in bread with thicker gluten matrix and lower viscoelasticity hence
137 increase bread hardness and decrease bread's springiness.

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

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

145 3.3 Sensory evaluation

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

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

158 4. Conclusion

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

164

165 **Conflict of interest**

166 The authors declare no conflict of interest.

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170 contract number 130X/WM01.5/N/2020.

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243

244 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

245 B₀ : 0% bee pollen; B₁ : 0.075% bee pollen; B₂ : 0.150% bee pollen; B₃ : 0.225% bee pollen; B₄ : 0.300% bee
 246 pollen; B₅ : 0.375% bee pollen

247

248 Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented
 249 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

250 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 251 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 252 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 253 bee pollen

254

255 Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented
 256 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

257 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 258 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 259 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 260 bee pollen

261

262

263

264 Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented
 265 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

266 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 267 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 268 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 269 bee pollen

270
 271
 272 Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and
 273 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

274 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 275 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 276 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 277 bee pollen

278 Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian
 279 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

280 Values are presented as mean±SD (n = 50). Values with the same superscript within column are not
 281 significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 282 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 283 bee pollen

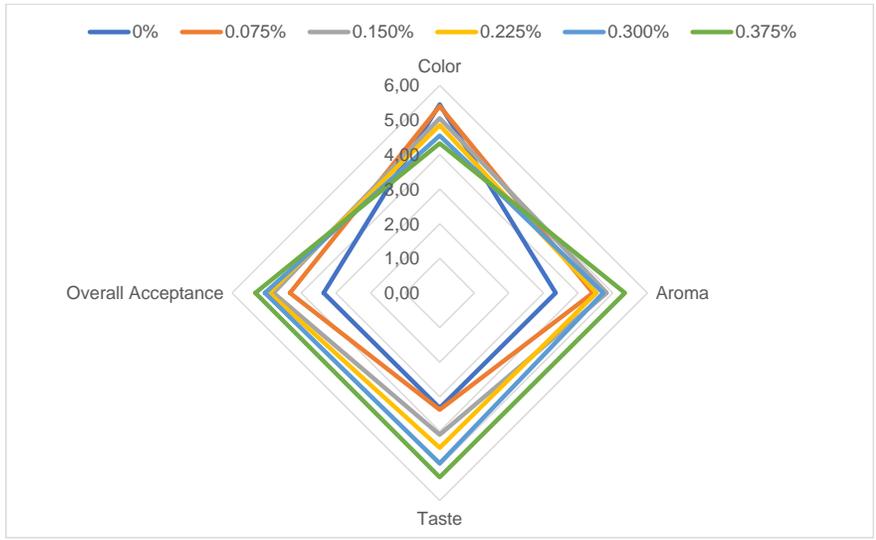


Figure 1. Spider web test result

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

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14

15 **Abstract**

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

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

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

28 1. Introduction

29 The development of functional bakery products had been widely studied among food scientists
30 as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.*
31 (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented
32 durian seeds (MFDS) into bread. Rice bran contains γ -orizanol, γ -tocotrienol and fibre which can regulate
33 blood sugar levels (Premakumari *et al.*, 2013; Sivamaruthi *et al.*, 2018). MFDS contains monascin pigments
34 that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production.
35 (Nugerahani *et al.*, 2017; Faroukh and Baumgärtel, 2019).

36 Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and
37 taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains
38 monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty
39 acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as
40 tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.*,
41 2016; Hasim *et al.*, 2019).

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

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

50 **2. Materials and methods**

51 *2.1 Materials*

52 Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice
53 bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table
54 salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima"
55 multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which
56 was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University
57 Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture
58 of *Monascus purpureus* M9, Ca(OH)₂, distilled water and potato dextrose agar (Merck 1.10130.0500).

59 *2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran*

60 Sample preparation began by mixing all of the dry ingredients except table salt for 1 min and
61 followed by adding water. The mixing process then continued for 10 mins. Margarine and table salt
62 was then added and the mixing process continued for 5 mins. The dough was fermented at 26°C for
63 30 mins. After the dough was shaped into a loaf the dough was proofed at 26°C for 90 mins. The bread
64 was baked with the oven at 180°C for 30 mins then cooled at 26°C for 60 mins. Table 1 shows the
65 composition of bread incorporated with MFDS and RB with different levels of bee pollen
66 concentration.

67 *2.3 Monascus-fermented durian seeds preparation*

68 Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that,
69 the durian seeds were boiled with 5% Ca(OH)₂ solution at 85-90°C for 10 mins [durian seeds: 5%

70 Ca(OH)₂ solution = 1:1 (w/v)]. The durian seeds were removed from the Ca(OH)₂ solution and then
71 washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through
72 first drying process at 45°C for 40 mins. The durian seeds were then weighed into 50 g. After that,
73 durian seeds were sterilized at 121°C, 15 lbs/inch² for 10 mins and cooled down at 26°C for 30 mins.
74 The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were
75 put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at
76 45°C for 24 h, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.*,
77 2016).

78 2.3 Moisture content analysis

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

$$82 \text{ Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)} - \text{weight loss (g)}} \times 100\%$$

83 2.4 Specific volume

84 Specific volume determination was carried out using seed displacement method according to
85 Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds
86 which were above the container rim were then removed using straight ruler. All the foxtail millet
87 seeds in the container were then poured into a measuring cylinder to measure the volume of the
88 container (V₁). These steps were then repeated except that a loaf of bread sample was already inside
89 the container before it was filled with seeds to obtain V₂.

$$90 \text{ Specific volume (cm}^3/\text{g)} = \frac{V_1 \text{ (ml)} - V_2 \text{ (ml)}}{W \text{ (g)}}$$

91

92

93 *2.5 Texture analysis*

94 Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out
95 using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential
96 compression events (probe: P36/R, pre-test speed: 5 mm s⁻¹, test speed: 1.5 mm s⁻¹, post-test speed
97 10 mm s⁻¹, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

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

102 *2.6 Color analysis*

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

106 *2.8 Sensory evaluation*

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

113 *2.9 Statistical analysis*

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

117 3. Results and discussion

118 3.1 Moisture Content

119 According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture
120 content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that
121 maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice
122 bran contains 7-11% fiber (Henderson *et al.*, 2012). Ability of fiber to bind with water then resulted
123 in increased moisture content of bread (Sangle *et al.*, 2017). Bee pollen contains glucose, fructose and
124 sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*,
125 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were
126 hydrophilic amino acid (Taha *et al.*, 2017). Conte *et al.* (2020) also reported that moisture content of
127 gluten free bread increased as bee pollen concentration increased.

128 3.2 Physical properties

129 Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due
130 to additional sugar from bee pollen which are useful CO₂ production by yeast. The CO₂ pushed the
131 gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO₂ gas
132 also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-
133 0.375% bee pollen showed a decrease in specific volume due to competition in binding water between
134 the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the
135 gluten matrix became weaker and could not retain CO₂ as much as bread with 0-0.15%. Lower
136 retention of CO₂ also resulted in bread with thicker gluten matrix and lower viscoelasticity hence
137 increase bread hardness and decrease bread's springiness.

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

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

145 3.3 Sensory evaluation

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

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

158 4. Conclusion

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

164

165 **Conflict of interest**

166 The authors declare no conflict of interest.

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243

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244 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

245 B₀ : 0% bee pollen; B₁ : 0.075% bee pollen; B₂ : 0.150% bee pollen; B₃ : 0.225% bee pollen; B₄ : 0.300% bee
 246 pollen; B₅ : 0.375% bee pollen

247

248 Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented
 249 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

250 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 251 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 252 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 253 bee pollen

254

255 Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented
 256 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

257 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 258 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 259 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 260 bee pollen

261

262

263

264 Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented
 265 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

266 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 267 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 268 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 269 bee pollen

270
 271
 272 Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and
 273 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

274 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 275 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 276 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 277 bee pollen

278 Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian
 279 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

280 Values are presented as mean±SD (n = 50). Values with the same superscript within column are not
 281 significantly ($p > 0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 282 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 283 bee pollen

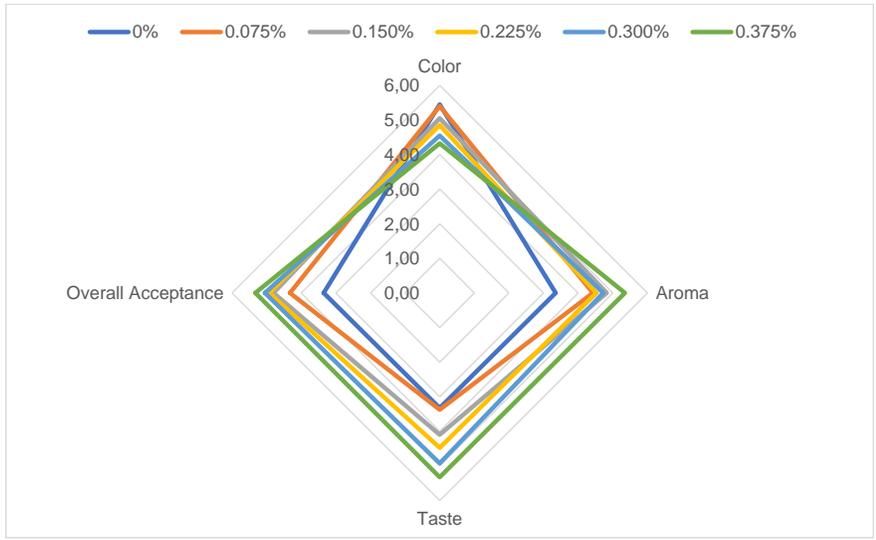


Figure 1. Spider web test result

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

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14

15 **Abstract**

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

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

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

28 1. Introduction

29 The development of functional bakery products had been widely studied among food scientists
30 as an approach to consumer's demand for baked products with extra health benefits. Trisnawati *et al.*
31 (2019) studied the effect of adding functional ingredients such as rice bran (RB) and *Monascus*-fermented
32 durian seeds (MFDS) into bread. Rice bran contains γ -orizanol, γ -tocotrienol and fibre which can regulate
33 blood sugar levels (Premakumari *et al.*, 2013; Sivamaruthi *et al.*, 2018). MFDS contains monascin pigments
34 that can help to reduce blood sugar level and monacolin-K which helps to reduce cholesterol production.
35 (Nugerahani *et al.*, 2017; Faroukh and Baumgärtel, 2019).

36 Incorporation of MFDS flour and RB flour however reduce the preference score for aroma and
37 taste of bread incorporated with MFDS and RB as reported by Trisnawati *et al.* (2019). RB contains
38 monounsaturated fatty acid and polyunsaturated fatty acid which are easily oxidized into short chain fatty
39 acids which have rancid properties (Cho and Samuel, 2009). MFDS contain secondary metabolites such as
40 tannin and alkaloid also produced and therefore resulted in bitterness and astringency (Reginio *et al.*,
41 2016; Hasim *et al.*, 2019).

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

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

50 **2. Materials and methods**

51 *2.1 Materials*

52 Materials that were used for making bread incorporated with MFDS and RB were bread flour, rice
53 bran flour, instant dry yeast, instant full cream milk powder, granulated sugar, bread improver, table
54 salt, mineral water, margarine which were purchased from local distributor, "Mirah Delima"
55 multiflora bee pollen which was purchased from Mirah Delima Bee Farm and also MFDS flour which
56 was produced in Laboratory of Food Industrial Microbiology, Widya Mandala Catholic University
57 Surabaya. Materials that were used for producing MFDS flour were Petruk durian seeds, pure culture
58 of *Monascus purpureus* M9, Ca(OH)₂, distilled water and potato dextrose agar (Merck 1.10130.0500).

59 *2.2 Preparation of bread incorporated with Monascus-fermented durian seeds and rice bran*

60 Sample preparation began by mixing all of the dry ingredients except table salt for 1 min and
61 followed by adding water. The mixing process then continued for 10 mins. Margarine and table salt
62 was then added and the mixing process continued for 5 mins. The dough was fermented at 26°C for
63 30 mins. After the dough was shaped into a loaf the dough was proofed at 26°C for 90 mins. The bread
64 was baked with the oven at 180°C for 30 mins then cooled at 26°C for 60 mins. Table 1 shows the
65 composition of bread incorporated with MFDS and RB with different levels of bee pollen
66 concentration.

67 *2.3 Monascus-fermented durian seeds preparation*

68 Preparation of MFDS flour began with sortation. Durian seeds then washed with water. After that,
69 the durian seeds were boiled with 5% Ca(OH)₂ solution at 85-90°C for 10 mins [durian seeds: 5%

70 Ca(OH)₂ solution = 1:1 (w/v)]. The durian seeds were removed from the Ca(OH)₂ solution and then
71 washed with water. The durian seeds were then cut into 1 cm x 1 cm x 1 cm cubes and went through
72 first drying process at 45°C for 40 mins. The durian seeds were then weighed into 50 g. After that,
73 durian seeds were sterilized at 121°C, 15 lbs/inch² for 10 mins and cooled down at 26°C for 30 mins.
74 The sterilized durian seeds then inoculated with *Monascus purpureus* M9 starter (5% v/w) and were
75 put under aerobic fermentation at 30±1°C for 14 days to produce MFDS. The MFDS were dried at
76 45°C for 24 h, grinded and then sifted with 40 mesh sifters to get MFDS flour (Puspitadewi *et al.*,
77 2016).

78 2.3 Moisture content analysis

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

$$82 \text{ Moisture content (\%)} = \frac{\text{sample weight (g)}}{\text{sample weight (g)} - \text{weight loss (g)}} \times 100\%$$

83 2.4 Specific volume

84 Specific volume determination was carried out using seed displacement method according to
85 Nwosu *et al.* (2014). An empty container was filled with foxtail millet seeds until overflowed and seeds
86 which were above the container rim were then removed using straight ruler. All the foxtail millet
87 seeds in the container were then poured into a measuring cylinder to measure the volume of the
88 container (V₁). These steps were then repeated except that a loaf of bread sample was already inside
89 the container before it was filled with seeds to obtain V₂.

$$90 \text{ Specific volume (cm}^3/\text{g)} = \frac{V_1 \text{ (ml)} - V_2 \text{ (ml)}}{W \text{ (g)}}$$

91

92

93 *2.5 Texture analysis*

94 Prior to analysis, bread samples were cut into 12 mm thickness. Texture analysis was carried out
95 using texture analyzer (TA-XT Plus, Stable Micro System) and performed by two sequential
96 compression events (probe: P36/R, pre-test speed: 5 mm s⁻¹, test speed: 1.5 mm s⁻¹, post-test speed
97 10 mm s⁻¹, distance: 12 mm, time: 3 s, trigger type: auto, trigger force: 5 g).

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

102 *2.6 Color analysis*

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

106 *2.8 Sensory evaluation*

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

113 *2.9 Statistical analysis*

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

117 3. Results and discussion

118 3.1 Moisture Content

119 According to Table 2., incorporation of bee pollen to bread with MFDS and RB resulted in moisture
120 content ranging from 40.29% to 59.25% while Badan Standarisasi Nasional (1995) suggested that
121 maximum moisture content of bread is 40% to support shelf-life against microbial deterioration. Rice
122 bran contains 7-11% fiber (Henderson *et al.*, 2012). Ability of fiber to bind with water then resulted
123 in increased moisture content of bread (Sangle *et al.*, 2017). Bee pollen contains glucose, fructose and
124 sucrose which were able to form hydroxyl bond with water molecules (Komosinska-Vassev *et al.*,
125 2013). that bee pollen is high in lysine, arginine, cysteine, tryptophan, and tyrosine which were
126 hydrophilic amino acid (Taha *et al.*, 2017). Conte *et al.* (2020) also reported that moisture content of
127 gluten free bread increased as bee pollen concentration increased.

128 3.2 Physical properties

129 Increase of specific volume in bread samples with 0-0.15% bee pollen as seen in Table 3 was due
130 to additional sugar from bee pollen which are useful CO₂ production by yeast. The CO₂ pushed the
131 gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO₂ gas
132 also increased viscoelasticity of gluten matrix hence springiness increased. Bread samples with 0.225-
133 0.375% bee pollen showed a decrease in specific volume due to competition in binding water between
134 the hydrophilic molecules (protein and sugar) in bee pollen and gluten forming protein. Hence the
135 gluten matrix became weaker and could not retain CO₂ as much as bread with 0-0.15%. Lower
136 retention of CO₂ also resulted in bread with thicker gluten matrix and lower viscoelasticity hence
137 increase bread hardness and decrease bread's springiness.

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

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

145 3.3 Sensory evaluation

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

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

158 4. Conclusion

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

164

165 **Conflict of interest**

166 The authors declare no conflict of interest.

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243

244 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

245 B₀ : 0% bee pollen; B₁ : 0.075% bee pollen; B₂ : 0.150% bee pollen; B₃ : 0.225% bee pollen; B₄ : 0.300% bee
 246 pollen; B₅ : 0.375% bee pollen

247

248 Table 2. Effect of bee pollen on the moisture content of bread incorporated with *Monascus*-fermented
 249 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

250 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 251 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 252 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 253 bee pollen

254
 255 Table 3. Effect of bee pollen on the specific volume of bread incorporated with *Monascus*-fermented
 256 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

257 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 258 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 259 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 260 bee pollen

261
 262
 263

264 Table 4. Effect of bee pollen on textural properties for bread incorporated with *Monascus*-fermented
 265 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

266 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 267 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 268 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 269 bee pollen

270

271

272 Table 5. Effect of bee pollen on color of bread incorporated with *Monascus*-fermented durian seeds and
 273 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

274 Values are presented as mean±SD (n = 4). Values with the same superscript within column are not
 275 significantly (p>0.05) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 276 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 277 bee pollen

278 Table 6. Effect of bee pollen on preference score for bread incorporated with *Monascus*-fermented durian
 279 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

280 Values are presented as mean±SD (n = 50). Values with the same superscript within column are not
 281 significantly ($p>0.05$) different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$), B₀: 0% bee pollen,
 282 B₁: 0.075% bee pollen, B₂: 0.150% bee pollen, B₃: 0.225% bee pollen, B₄: 0.300% bee pollen, and B₅: 0.375%
 283 bee pollen

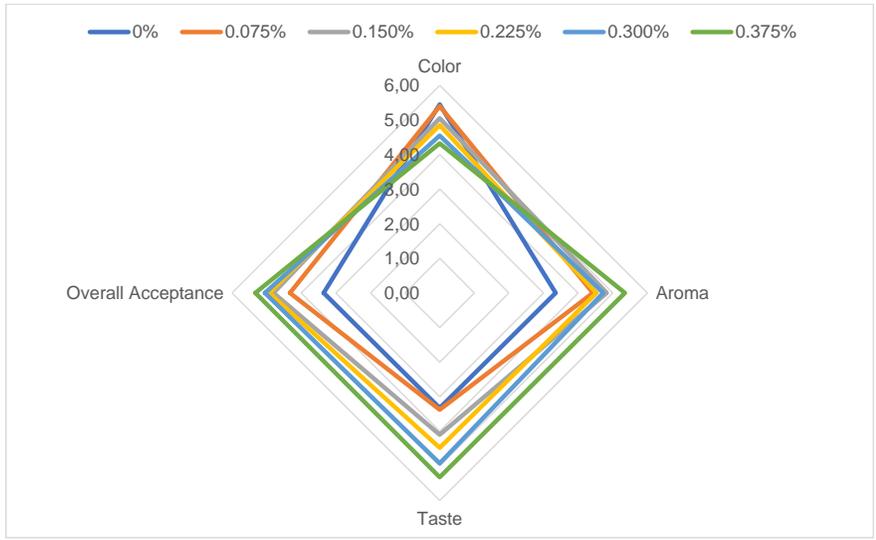


Figure 1. Spider web test result

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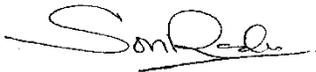
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Manuscript Title : Effect of bee pollen on the characteristic of bread incorporated with Monasus-fermented durian seeds and rice bran

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

We thank you for your fine contribution to the Food Research journal and encourage you to submit other articles to the Journal.

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

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

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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 -orizanols, γ -tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.*, 2013; Sivamaruthi *et al.*, 2018). MFDS contains monascins 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 ketone (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/inch² 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:

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

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 (V₁). 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 V₂.

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

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

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

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.

lightness (L^*), redness (a^*), yellowness (b^*), chroma ($*C$) and hue ($^{\circ}H$) 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 $\alpha = 5\%$ 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

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 ($\alpha = 0.05$). 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.

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 CO₂ production by yeast. The CO₂ pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO₂ 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 CO₂ as much as bread with 0-0.15%. Lower retention of CO₂ 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 ^c
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 ($\alpha = 0.05$). 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 ($\alpha = 0.05$). 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 ^c
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 ($\alpha = 0.05$). 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 ($\alpha = 0.05$). 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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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 -orizanols, γ -tocotrienol and fibre which can regulate blood sugar levels (Premakumari *et al.*, 2013; Sivamaruthi *et al.*, 2018). MFDS contains monascins 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 ketone (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/inch² 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:

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

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 (V₁). 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 V₂.

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

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

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

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.

lightness (L^*), redness (a^*), yellowness (b^*), chroma ($*C$) and hue ($^{\circ}H$) 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 $\alpha = 5\%$ 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

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 ($\alpha = 0.05$). 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.

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 CO₂ production by yeast. The CO₂ pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO₂ 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 CO₂ as much as bread with 0-0.15%. Lower retention of CO₂ 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 ^c
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 ($\alpha = 0.05$). 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 ($\alpha = 0.05$). 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 ^c
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 ($\alpha = 0.05$). 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.

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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 = 4). Values with different superscripts within column are statistically significantly different evaluated by ANOVA followed by DMRT test ($\alpha = 0.05$). 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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Effect of bee pollen on the characteristic of bread incorporated with *Monascus*-fermented durian seeds and rice bran

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

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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 -orizanols, 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/inch² 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:

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

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 (V₁). 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 V₂.

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

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

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.

Color analysis was carried out by measuring the lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*) and hue ($^{\circ}H$) 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 $\alpha = 5\%$ 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

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 ($\alpha = 0.05$). 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.

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.

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 CO₂ production by yeast. The CO₂ pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO₂ 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 CO₂ as much as bread with 0-0.15%. Lower retention of CO₂ 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 ($\alpha = 0.05$). 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 ^c	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 ^c	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 ($\alpha = 0.05$). 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 ^c
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 ($\alpha = 0.05$). 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 pyrrol 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

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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 ($\alpha = 0.05$). 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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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 -orizanols, 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/inch² 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:

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

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 (V₁). 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 V₂.

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

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

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.

Color analysis was carried out by measuring the lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*) and hue ($^{\circ}H$) 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 $\alpha = 5\%$ 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

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 ($\alpha = 0.05$). 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.

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.

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 CO₂ production by yeast. The CO₂ pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO₂ 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 CO₂ as much as bread with 0-0.15%. Lower retention of CO₂ 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 ($\alpha = 0.05$). 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 ^c	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 ^c	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 ($\alpha = 0.05$). 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 ^c
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 ($\alpha = 0.05$). 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 pyrrol 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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References

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 ($\alpha = 0.05$). 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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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/inch² 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:

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

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 (V₁). 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 V₂.

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

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

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.

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 $\alpha = 5\%$ 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

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 ($\alpha = 0.05$). 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.

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.

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 CO₂ production by yeast. The CO₂ pushed the gluten matrix to become thinner and resulted in reduced hardness as seen in Table 4. More CO₂ 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 CO₂ as much as bread with 0-0.15%. Lower retention of CO₂ 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 ($\alpha = 0.05$). 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 ($\alpha = 0.05$). 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 ^c
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 ($\alpha = 0.05$). 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 pyrrol 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 ($\alpha = 0.05$). 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.

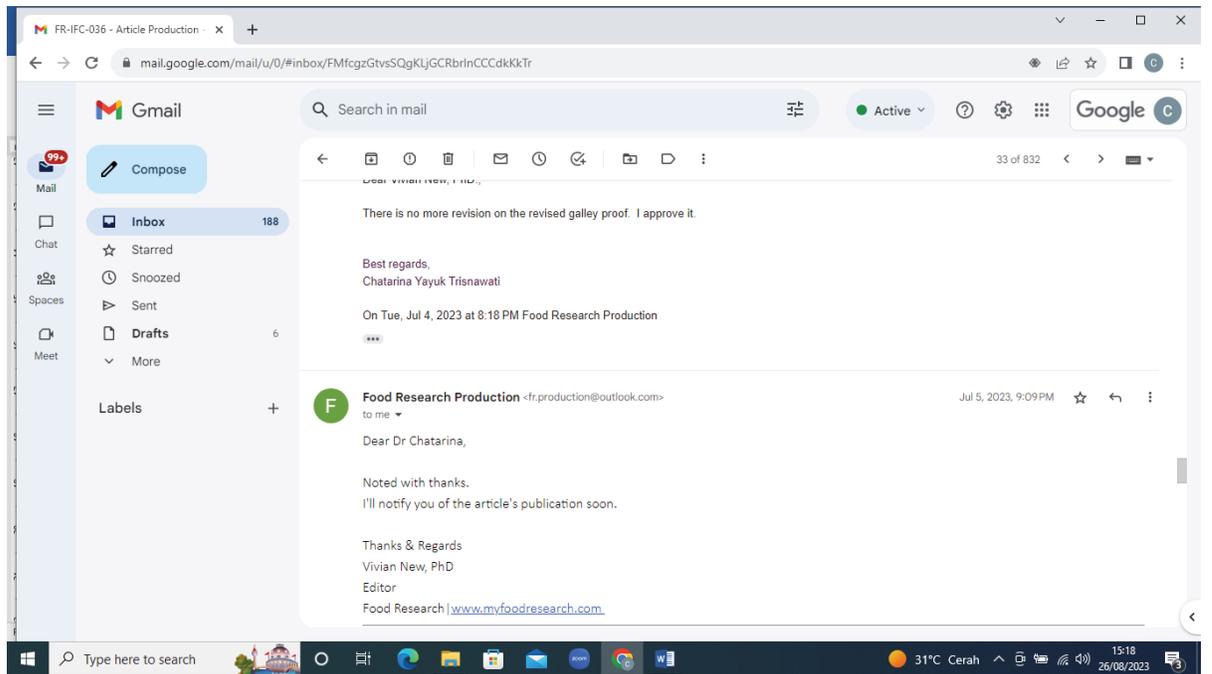
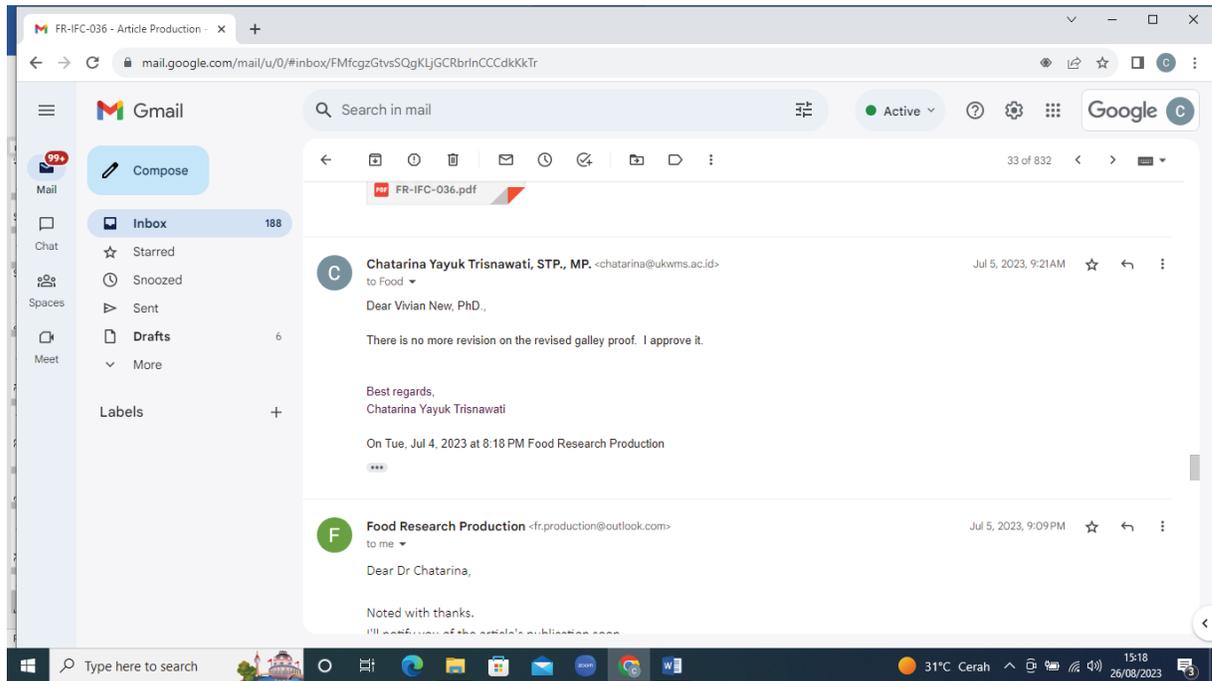
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