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FULL PAPER

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

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Abstract

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

1. Introduction

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

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

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

2. Materials and methods

2.1 Materials

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

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

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

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

2.3 *Monascus*-fermented durian seeds preparation

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

2.4 Moisture content analysis

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

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

2.5 Specific volume

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

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

2.6 Texture analysis

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

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

2.7 Color analysis

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

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

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

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

2.8 Sensory evaluation

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

2.9 Statistical analysis

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

3. Results and discussion

3.1 Moisture content

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

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

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

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

3.2 Physical properties

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

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

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

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

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

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

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

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

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

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

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

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

3.3 Sensory evaluation

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

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

4. Conclusion

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

Conflict of interest

The authors declare no conflict of interest.

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AOAC (Association of Official Analytical Chemists).

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

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

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

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