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Effect of larval-stage mealworm (*Tenebrio molitor*) powder on qualities of bread

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Abstract Wheat flour without addition of larval-stage mealworm powder (control) had the highest peak viscosity of 139.14 RVU. The addition of higher levels of larval-stage mealworm powder resulted in significantly decrease in peak viscosity of wheat flour ($p<0.05$). The pasting temperatures of all samples were between 87.20 and 89.50^{o}C. The hardness of bread without the addition of larval-stage mealworm powder was 108.50 g_{force}, whereas the hardness of fortified bread samples raised approximately 4 times when adding larval-stage mealworm powder to 5-15% (425.83 to 487.67 g_{force}). It was found that an increase of larval-stage mealworm powder leading to lower specific volume and higher hardness in bread after baking. The fortification of larval-stage mealworm powder also caused the increase of color intensity due to the original brown color of larval-stage mealworm powder and the occurrance of maillard reaction during baking. The non-uniformity of porosity of the bread products was observed in fortified bread samples. The bread products added by larval-stage mealworm powder at 0, 5, 10 and 15% of wheat flour had protein contents of 9.63, 12.63, 13.21 and 13.73% respectively. The bread products fortified at 5% of wheat flour revealed the most comparable quality to the control bread sample.

Keywords: Bread, Insect, Mealworm, Protein fortification

Introduction

Population growth rate is increasing and leaded to high resource consumption, requiring a large amount of the agriculture land, water and energy for Livestock production. Alternative protein sources which can be produced using fewer resources and provided higher productivity are seeked (Jansson and Berggren, 2015; Hartmann and Siegrist, 2016).

Edible insects have become meat alternatives of healthy protein, fat, dietary fibre and useful quantities of important micronutrients, potentially low environmental and land use impacts (Smetana *et al*., 2018). Some edible insects

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such as cricket, silkworm and palm weevil show their nutritional values that can be compared with meat and fish, while others; for example, mopane worm and mealworm have higher proportion of proteins, fat, and energy values. (Payne *et al.*, 2015; Tiencheu and Womeni, 2017). Mealworm (*Tenebrio molitor*) is edible insect which are eaten in Africa, Asia, Australia and America (Alves *et al.*, 2016). Mealworms are often used as an alternative protein-rich in animal feed, but they are not only suitable for animal feed but also considered as food for human (Grau *et al.*, 2017). Amount of studies relate to applications of mealworm in human foods have been reported. In cereal product, mealworms were added into tortilla, resulting to an increase of protein content (Aguilar-Miranda *et al*., 2002). Muffins enriched with 8% of mealworm were found to be acceptable for consumers (Hwang and Choi, 2015). In meat product, 10% of mealworm was found to increase cooking yield and hardness of emulsion sausage (Kim *et al.*, 2016). Fusion of mealworm and beef in burgers was acceptable in term of taste and acceptability (Megido *et al.*, 2016). Replacement of lean pork with 10% of mealworm in frankfurters resulted in the level of quality similar to regular control (Choi *et al.*, 2017). All of these reports confirmed the possibility of using mealworm as ingredient in human food. The researchers also found that consumers would be willing to eat insect in a less visible form, so insects could be processed as a powder (González *et al*., 2019). Moreover, insects as ingredients in the form of powder are easier to be applied in food.

Bread is a leavening product made from wheat flour, water, salt, yeast and other ingredients. Bread is the most important and usually consumes food in the world because it contained high amount of carbohydrate, therefore it can be an energy source (de Oliveira *et al.*, 2017; Osimani *et al*., 2018). The excessive of carbohydrate consumption has a negative effect on health (Neacşu, 2014). Consequently, protein sources from animal and plant for enrichment of bread have been studied. Cakmak *et al.* (2013) are enriched bread with chicken powder and found that the addition of chicken powder significantly affected the quality and sensorial characteristics of bread. Fagundes *et al*. (2018) found that fortification of cobia (*Rachycentron canadum*) in bread increased protein content, but reduced bread qualities. Mohammed *et al*. (2014) observed that mixing of chickpea could improve protein content of bread.

The investigation of protein enrichment in wheat bread was least studied by using insects as alternative protein source. Therefore, the purposes of research finding were to study the addition of mealworm powder into wheat bread in order to enrich nutrition values and to determine the quality of mealworm fortified bread products.

Materials and Methods

Mealworm powder preparation

Mealworm powder was provided by Department of Biology, Faculty of Science, Mahidol University, Thailand. The mealworm beetles at larval stage were unfed for 2 days before powder production. Mealworms were processed and dried by freeze drying method and then grounded into powder. Mealworm powder was packed in aluminium bags.

Bread loaves production

The experiment was run during the month of January and April in a laboratory at Thammasat University, Pathum Thani, Thailand. Wheat flour (UFM Food Centre Co., Ltd., Bangkok, Thailand), instant dried yeast (KCG Corporation Co., Ltd., Bangkok, Thailand), sugar (Thai Roong Ruang industry Co., Ltd., Phetchaburi, Thailand), salt (Saha Pathanapibul Pub Co., Ltd., Bangkok, Thailand), shortening (Lam Soon Co., Ltd., Bangkok, Thailand), egg (Central Food Retail Co., Ltd., Pathumthani, Thailand) and milk (Dutch Mill Co., Ltd., Bangkok, Thailand) were perchased from supermarkets nearby Thammasat University, Thailand. The bread formula was 55% wheat flour, 1% instant dried yeast, 3% sugar, 0.15% salt, 6% shortening, 2% egg yolk, 6% milk and 30% water. The sponge method was used to produce bread loaf. Half of the total amount of wheat flour and water of the formulation were mixed with instant dried yeast to produce sponge. All sponge ingredients were mixed and left for 1 hour. The sponge was homogenized with other ingredients using a food mixer machine (Spar mixer, 800-B, Taiwan) for 30 minutes. The dough was rounded and rested in a mold covered with wet cloth in order to maintain relative humidity of dough. The dough was baked in the oven (Union Progress, UP-AG100, Italy) at 180^{o}C for 20 minutes. After that, bread loaf was cooled to room temperature. For bread-making studies, three levels of mealworm powder (5, 10 and 15% of wheat flour) were used to produce mealworm fortified bread loaves whereas a control sample without the addition of mealworm powder was also baked.

Quality determination

Protein contents of mealworm powder and all bread products were analyzed according to Kjeldahl method (AOAC 992.23, 2016) by The Institute of Nutrition, Mahidol University, Thailand. Fat and ash contents of mealworm powder were analysed according to AOAC (1990) by Department of Food

Science and Technology, Faculty of Science and Technology, Thammasat University, Thailand.

Pasting properties of wheat flour were analyzed using Rapid Viscosity Analyzer (Perten instuments, Tecmaster, Sweden). Wheat flour (control) and wheat flour mixed with mealworm powder at three various levels (5, 10 and 15%). Three grams of moisture content known sample were directly weighed into aluminum RVA canisters and distilled water was added (weight of distilled water received from program calculation depending on moisture content of each sample). The sample was heated to 50^{o}C and held for 1 min. Then the sample was heated up to 95^{o}C and held at that temperature for 2.5 min. And the sample was cooled down to 50^{o}C (Phuphechr, 2009). Values obtained from the pasting profile were pasting temperature (temperature that starch granules being to swell), peak viscosity (maximum paste viscosity), breakdown viscosity (difference between maximum viscosity and minimum viscosity), final viscosity (viscosity at the end of the run), and setback viscosity (difference between final viscosity and minimum viscosity).

Dough stickiness values were measured by using a texture analyzer (plus-upgrade, Stable Micro System, USA). Chen-Hoseney Dough Stickiness Rig test was applied to analyze dough stickiness with a 25 mm perspex cylinder probe (P/25P) and SMS/Chen-Hoseney Dough Stickiness cell (Chen and Hoseney, 1995). The screw was rotated to increase chamber space. Ten grams of prepared dough were placed into chamber and excess dough was removed with a spatula. The screw was rotated to extrude dough through the holes and first extrusion was removed from the lid using a spatula. The screw was rotated once again to extrude 1 mm high dough and perspex cylinder probe was placed over the sample surface under the following condition: Pre-Test speed: 0.5 mm/s, Test speed: 0.5 mm/s, Post-Test-speed: 10.0 mm/s, Distance: 4 mm, Force: 40 g, Time: 0.1 s, Trigger type: Auto-5 g. The result obtained from the test was a force versus time curve. The positive maximum force in the curve is an indicator of dough stickiness (g).

Bread crumb texture analysis was measured on uniform slices of 25 mm thickness. Three slice samples from the center of each loaf were taken for evaluation. Texture profile analysis (TPA) was performed using a texture analyzer (plus-upgrade, Stable Micro System, USA) which was equipped with a 25 kg load cell and 50 mm cylindrical probe. The texture analyses were performed by two sequential compression events under the following condition: Pre-Test speed: 1.00 mm/s, Test-speed: 1.00 mm/s, Post speed: 1.00 mm/s, Distance: 40% (Panyathitipong and Peeraphatchara, 2016). The averages of at least ten analyses were calculated. Hardness, springiness and chewiness were recorded from measurement.

Specific volume was measured by seed displacement method described by Panyathitipong and Peeraphatchara (2016). Bread loaf was placed in a container of known volume (1,600 cm^3). Sesame seeds were poured until bread sample was covered. Then sesame seeds volume was measured in a cylinder. Specific volume of bread was calculated following equation (1) and (2):

$$\text{Loaf volume (cm}^3\text{)} = \text{container volume - sesame seeds volume} \quad (1)$$

$$\text{Specific volume (cm}^3\text{/g)} = \frac{\text{loaf volume (cm}^3\text{)}}{\text{loaf weight (g)}} \quad (2)$$

The photographs of bread loaves and bread slices were taken by a digital camera over the black background and the photographs were saved in JPEG format. Bread loaves height was measured by using a scale.

Color of bread samples were determined by using a colorimeter (CX2678, Hunter Lab, USA). Bread samples were cut into small pieces and transferred to an optical glass cell. Glass cell with sample was placed on a base plate and covered with an opaque lid. The color of samples were measured in L*,a*and b* parameters which indicated the lightness, redness and yellowness.

Porosity of bread crumb was obtained from ImageJ analysis and applying the method of Scheuer *et al.* (2015). The image of sliced bread crumb was cropped into 400x400 pixels. Then the image was converted to 8-bit greyscale. The threshold of image was set to gain the best cell resolution for analysis. Data derived from the image analysis included: number of cells (cells) and mean cell area (mm^2). Cell density (cells/mm^2) was calculated using the following equation (3):

$$\text{Cell density (cells/mm}^2\text{)} = \frac{\text{Number of cells (cells)}}{\text{mean cell area (mm}^2\text{)}} \quad (3)$$

The experimental design for evaluate the quality of bread products were set up as Completely Randomized Design (CRD) with three replications. All data were analyzed using analysis of variance (ANOVA). Duncan test was applied to determine the difference between mean values at significant level of $p<0.05$.

Results

The chemical compounds and physical characteristics of mealworm powder were analysed. The protein, fat and ash contents of mealworm powder were 41.22±0.62%, 32.76±1.70 and 3.57±0.06, respectively. This dried powder contained low water activity, which was 0.185±0.005. Lightness (L*), redness (a*), and yellowness (b*) values of mealworm powder were 56.46±0.23, 4.93±0.03 and 20.38±0.09, correspondingly.

Pasting properties of wheat flour fortified with different levels of mealworm powder are shown in Table 1. The pasting temperature of samples were in the range of 87.20-89.50°C. It was noticed that pasting temperature increased with the percentage of added mealworm powder whereas the peak viscosity and breakdown viscosity decreased. The addition of mealworm powder also resulted in the increase of final and setback viscosity.

The effect of mealworm powder on specific volume of bread product are presented in Table 2. Specific volume of bread products tended to decline with the increase of mealworm powder. Bread loaf without addition of mealworm powder had a highest specific volume.

Table 1. Pasting properties of wheat flour and wheat flour mixed with mealworm powder 5, 10 and 15%

Mealworm powder (%)	**Pasting temperature (°C)**	**Pasting properties (RVU)**			
		Peak viscosity	breakdown	Final viscosity	Setback
0	$87.20^{a1/}\pm0.52$	$139.14^{a}\pm8.00$	$49.05^{a}\pm3.35$	$175.36^{a}\pm7.80$	$85.28^{b}\pm3.15$
5	$88.78^{b}\pm1.12$	$116.80^{b}\pm3.31$	$45.27^{b}\pm2.31$	$161.80^{b}\pm3.37$	$92.28^{b}\pm2.55$
10	$89.18^{b}\pm1.06$	$106.89^{c}\pm2.65$	$40.50^{c}\pm1.42$	$163.99^{b}\pm3.09$	$97.67^{ab}\pm2.84$
15	$89.50^{a}\pm0.31$	$99.39^{c}\pm2.49$	$31.00^{d}\pm0.50$	$170.22^{ab}\pm2.86$	$101.83^{a}\pm3.84$

1/: Different letters in the same column indicates that values are significantly different ($p < 0.05$).

Table 2. Specific volume of bread loaves with and without mealworm powder addition

Mealworm powder (%)	**Specific volume (cm^3/g)**
0	$4.13^{a1/}\pm0.19$
5	$3.38^{b}\pm0.05$
10	$2.40^{c}\pm0.22$
15	$2.03^{d}\pm0.35$

1/: Different letters indicates that values are significantly different ($p < 0.05$).

The dough stickiness and textural properties of bread containing different levels of mealworm powder resulted to appear dough stickiness which increased with the increasing level of mealworm powder (Table 3). Bread product without addition of mealworm powder had the lowest hardness and chewiness values, which were 108.50 g_{force} and 72.52 respectively, while the highest springiness was 0.93. On the other hand, hardness and chewiness values were highly significantly for the bread products fortified with mealworm powder ($p<0.05$).

Table 3. Dough stickiness and textural properties of bread loaves with and without mealworm powder addition

Mealworm powder (%)	Dough stickiness (g)	TPA of bread		
		Hardness (g_{force})	Springiness	Chewiness
0	$41.37^{b}\pm3.52$	$108.50^{b1/}\pm15.47$	$0.93^{a}\pm0.01$	$72.52^{c}\pm9.58$
5	$47.70^{ab}\pm3.78$	$425.83^{a}\pm78.60$	$0.84^{b}\pm0.01$	$190.75^{b}\pm30.18$
10	$50.39^{ab}\pm3.94$	$483.99^{a}\pm78.90$	$0.83^{b}\pm0.02$	$199.77^{ab}\pm36.33$
15	$58.85^{a}\pm3.10$	$487.67^{a}\pm78.70$	$0.80^{c}\pm0.01$	$223.25^{a}\pm34.93$

1/: Different letters in the same column indicates that values are significantly different ($p< 0.05$).

The values of L* (lightness), a* (redness), b* (yellowness) of bread products are illustrated in Table 4. Breads fortified with mealworm powder presented a reduction in lightness (L*) and yellowness (b*), while raising in redness (a*). This indicated that fortification with mealworm powder caused the color intensity in bread products.

Table 4. Color values (L*, a* and b*) of crumb and crust of bread products

Mealworm powder (%)	Crumb			Crust		
	L*	a*	b*	L*	a*	b*
0	$61.04^{a1/}\pm1.10$	$0.69^{d}\pm0.19$	$15.54^{c}\pm0.78$	$61.42^{a}\pm2.99$	$2.97^{d}\pm0.91$	$21.70^{a}\pm1.97$
5	$59.12^{b}\pm1.06$	$4.21^{c}\pm0.16$	$21.36^{a}\pm3.05$	$42.01^{b}\pm0.51$	$7.81^{c}\pm0.63$	$19.84^{b}\pm0.72$
10	$47.50^{c}\pm1.38$	$5.32^{b}\pm0.21$	$18.04^{b}\pm0.35$	$37.62^{c}\pm0.47$	$8.28^{ab}\pm0.52$	$17.79^{c}\pm0.63$
15	$44.50^{d}\pm0.63$	$5.57^{a}\pm0.23$	$16.74^{cd}\pm0.72$	$34.39^{d}\pm0.35$	$8.86^{a}\pm0.46$	$16.37^{d}\pm1.11$

1/: Different letters in the same column indicates that values are significantly different ($p< 0.05$).

The cross section of breads revealed the appearance of bread crumb (Figure 1). It showed that bread products supplemented with higher level of mealworm powder had darker color, larger holes and denser crumb. Bread product without addition of mealworm powder had greater cell size and lighter color. For the height of bread loaves fortified with various mealworm powder contents, photographs showed that the height of bread loaves slightly decreased with an increase of mealworm powder addition. The minimum height was found in bread fortified with 15% mealworm powder. The reduction of bread height had a positive correlation with the values of specific volume of bread products.

The images of sliced bread crumb samples (control and 5, 10 and 15% mealworm powder addition formulas) were cropped (Figure 2(a-d)) and converted to grayscale (Figure 2(e-h)) for analysis their porosity by ImageJ program in term of number of cells, average size and cell density.

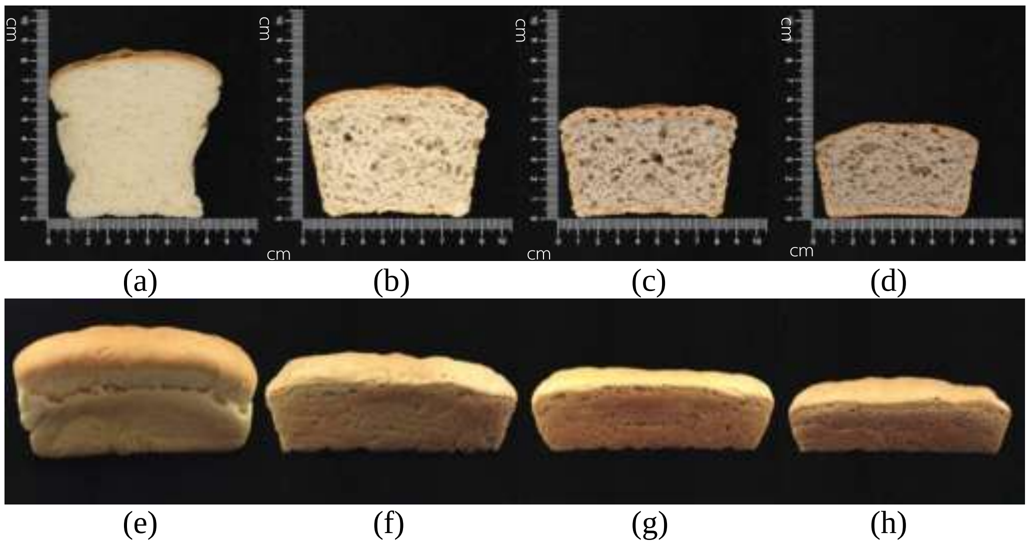

Figure 1. Cross sections of bread loaves containing 0% (a), 5% (b), 10% (c) and 15% (d) mealworm powder. Side view of bread loaves containing 0% (e), 5% (f), 10% (g) and 15% (h) mealworm powder.

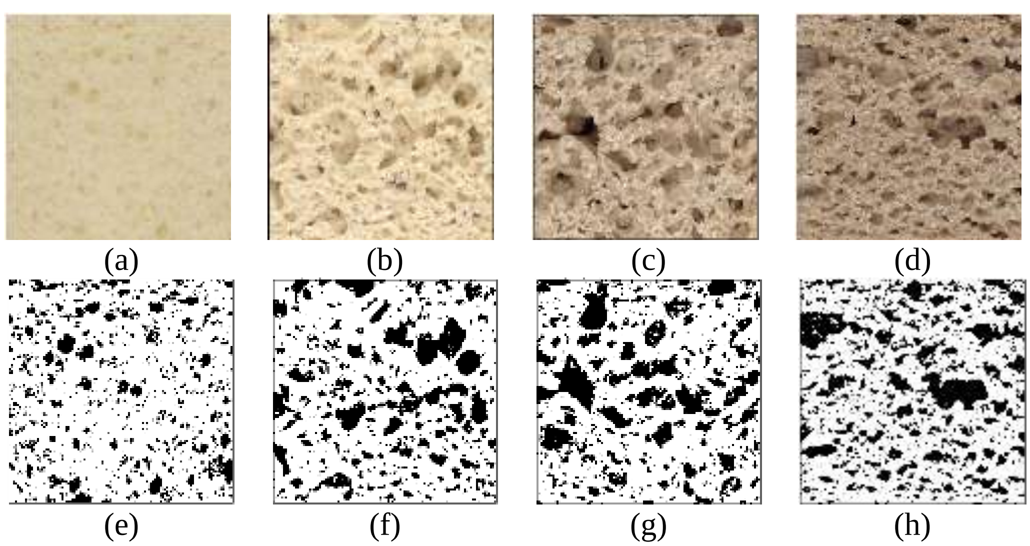

Figure 2. Photographs and graysacle image analysis of bread crump samples containing 0% (a,e), 5% (b,f), 10% (c,g) and 15% (d,h) mealworm powder

Number of cells, average size and cell density of bread cramb samples are shown in Table 5. Bread products supplemented with mealworm powder had significantly higher numbers of cells ($p<0.05$) than bread without mealworm addition. The average sizes of all bread products were found in the range of 2.66-6.90 mm^2. Cell density values deceased with the higher mealworm powder content. The maximum cell density was 17.43 cells/mm^2 which was found in bread without the addition of mealworm powder while the minimum cell density was 11.92 cells/mm^2 which was observed in bread fortified with 15% mealworm powder.

Table 5. Number of cells, average size and cell density of bread crumb samples

Mealworm powder (%)	Number of cells (cells)	Average size (mm^2)	Cell density (cells/mm^2)
0	$46.33^{c1/}\pm4.04$	$2.66^{c}\pm0.29$	$17.43^{a}\pm0.62$
5	$68.00^{b}\pm2.64$	$4.66^{b}\pm1.00$	$15.16^{ab}\pm3.93$
10	$71.00^{b}\pm5.29$	$5.13^{b}\pm0.09$	$13.83^{ab}\pm0.78$
15	$82.66^{a}\pm1.51$	$6.90^{a}\pm0.10$	$11.92^{b}\pm0.64$

1/: Different letters in the same column indicates that values are significantly different ($p< 0.05$).

The bread products were analyzed for protein content as shown in Table 6. It was found that protein content in mealworm bread formulas were higher than that of the control (0% mealworm) and significantly increased with the increasing proportion of mealworm powder ($p< 0.05$).

Table 6. Protein content of bread products

Mealworm powder (%)	Protein content (%)
0	$9.63^{a1/}\pm0.01$
5	$12.63^{b}\pm0.16$
10	$13.21^{c}\pm0.21$
15	$13.73^{d}\pm0.04$

1/: Different letters indicates that values are significantly different ($p< 0.05$).

Discussion

Protein, fat and ash contents of mealworm powder were averagred of 41.22, 32.76 and 3.57%, respectively. González *et al.* (2019) reported that protein, fat and ash content in mealworm were 48.82, 30.69 and 4.25% respectively that were similar to the result in the current study. Mealworm powder contained low content of water activity (0.185); therefore, it was safe from microbilogical risk for long term storage.

The addition of mealworm powder significantly influenced to the pasting properties of wheat flour. It was noticed that pasting temperature of wheat flour containing mealworm powders were significantly increased. This indicated that temperature that starch being to swell was interrupted by mealworm powder. The addition of mealworm powder also resulted in the decrease of peak and breakdown viscosity. It might be because the proteins in mealworm powder restricted the swelling power of starch granules. Another reason, mealworm powder contains high amount of fat, which can be formed amylose-lipid complex when starch and lipid are heated together (Blazek, 2008). Similarly to the study of Mohammed *et al*. (2014), pasting temperature of wheat flour increased with the addition of chickpea, which containing high amount of fat, while decreased the peak viscosity. On the other hand, final and setback viscosity significantly increased with the increasing level of mealworm powder. High level of final and setback viscosity indicates high level of retrogradation which occures at lower temperatures. Retrogradation of starch (staling of bread) is the main cause of the increase in hardness of bread products that influences to the shelf-life and consumer acceptance (Kong and Singh, 2011; Wang *et al*., 2015).

Specific volume of bread significantly decreased with an increase of mealworm powder content. The decrease of specific volume may be due to the interruption of gluten formation, resulting in the decrease of gas retention ability during proofing and baking. Villarino *et al*. (2016) reported that bread-making is sensitive to the addition of non-gluten ingredient into wheat flour related to disturbance of gluten development and reduction in gas production. Based on the study of González *et al*. (2019), the presence of insect flour reduced the specific volume of bread becasuse of the weakening of gluten.

de Oliveira *et al.* (2017) found a negative correlation between specific volume and hardness of bread. According to the results in Table 3, the hardness and chewiness of bread significantly increased in breads containing high amount of mealworm powder; whereas, the springiness of bread significantly decreased. This could be explained by the compression of gas cells, resulting in the increase of crumb hardness (de Oliveira *et al*., 2017). High level of hardness and chewiness of bread indicated the resistance to deformation and long time requirement to chew bread product before swallow (de Oliveira *et al*., 2017; González *et al*., 2019). The addition of mealworm powder also affected dough stickiness. The increase of dough stickiness might be caused by the addition of mealworm powder which leaded to the reduction of water adsorption (González *et al*., 2019) and the presence of excess water which promoted dough stickiness (Ahmed and Thomas, 2018). High level of dough stickiness had a negative effect on bread making. Sticky dough is difficult to process because it would be stuck on the equipment surface, resulting in the decrease of bread quality.

The color of bakery product is an important factor for consumer acceptance. The bread crumb showed a lighter color than bread crust because the bread crumb was protected by bread crust from direct heating (Jusoh *et al*., 2008). The changes in color (L*, a* and b*) of bread loaves could be related to maillard reaction. Maillard reaction is the chemical reaction between reducing sugar and protein which occurs during heat processing leading to brown color development (Tamanna and Mahmood, 2015). High protein content in mealworm powder (41.22%) was possible to react, leading to browning pigment formation in bread. Another possible reason might be the natural color of mealworm powder. González *et al*. (2019) reported that the color of bakery product directly depends on the color of raw material. Mealworm powder had light brown color which caused an intensive color of bread products. Moreover, Hwang and Choi (2015) demonstrated that the fortification of mealworm in muffin resulted in a reduction of lightness (L*) and yellowness (b*), while the redness (a*) increased.

Higher concentration of mealworm powder in bread formulation produced large holes and dense crumb. Encina-Zelada *et al.* (2019) reported that bread loaves with greater cell size are preferred than compact, denser and closer crumbs in a consumer viewpoint. The fortification of mealworm powder in bread products resulted in the decrease of height of bread loaves. This phenomenon might be related to gas retention ability of gluten which had a positive correlation to specific volume. The height and specific volume of bread products containing mealworm powder (5-15%) were lower than those of the control (0% mealworm) which might be the reason for the harder bread crumb.

The addition of mealworm powder resulted in higher number of cells, larger of cell size and thicker of cell wall. The explaination is that the weakening of gluten occurred when mealworm powder was fortified in bread-making leading to the occurences of non-uniform air cells and coarsening of bread structure (Rosell and Gómez, 2007). This effect also resulted in the lower volume of bread, which related to specific volume values. Rathnayak *et al*. (2018) reported that the higher value of cell density indicates a finer bread structure. In this study, higher mealworm concentration caused the reduction of cell density because many gas cells were coalesced into the large cell. The thickness of gas cell wall directly related to the hardness of bread (Furlán *et al.*, 2015). Bread samples containing 5-15% mealworm powder had thicker cell wall as shown in Figure 2.

A positive correlation between protein content and amount of mealworm powder was found. The increase of protein content in bread should be due to the initial high protein content of mealworm powder (41.22%). The results are in agreement with the report of Choi *et al.* (2017) and Kim *et al*. (2016). They reported that the fortification of mealworm in frankfurters and emulsion sausages helped improving the protein content in their products. Therefore, the result in this study demonstrated that mealworm powder can be used as an ingredient in bread product for raising the protein content.

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