Physicochemical properties of Eri pupae flakes and their antibacterial activity against Salmonella sp., Escherichia coli and Staphylococcus aureus

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ABSTRACT

Aims: The objective of this study was to evaluate the physicochemical properties of Eri silkworm (Samia cynthia ricini) pupae flour, specifically the pH value, water activity, water content, fat value, ash value and protein value. The study was also aimed to determine the antibacterial activity of Eri silkworm pupae flour against Salmonella sp., Escherichia coli and Staphylococcus aureus.

Methodology and results: Pupae meal is a flour made from the extract of the Eri silkworm pupae used as a flour substitute in producing pupae flakes. Pupae meal had two particle size treatments, 60 and 80 mesh, whereas pupae flake had three pupae meal substitution treatments of 0%, 4% and 8%. The physicochemical properties, antioxidant activity and microbiology of pupae meal and flake were analyzed. The results showed that Eri pupae flour met the Indonesia National Standard for Cereals (SNI 01-4270-1996) requirements for food ingredients regarding pH value, water activity, water content, fat value and protein value. The flour also exhibited antibacterial activity against all three bacterial species tested, with S. aureus being the most susceptible. Antioxidant activity and capacity increased as the percentage of pupae meal in the pupae flakes increased. The IC₅₀ value of pupae meal, 68 µg/mL, was included in the strong category. Salmonella sp. were not found in the pupae meal and flake. Total plate count, E. coli and S. aureus numbers remained within food tolerance limits based on the Indonesia National Standard in both pupae flour and pupae flakes.

Conclusion, significance and impact of study: The particle size of pupae flour did not affect its physicochemical properties or antioxidant activity, and microbiologically, it met the Indonesia National Standard for flour. Moreover, the flakes product with pupae flour substitution influenced its physicochemical properties, with the best level at 8% pupae flour for the highest protein content and increased the flakes product's activity and antioxidant capacity. Based on the microbiological analysis of pupae flakes products, the microbial content in the pupae was still within safe limits for human consumption. Research findings suggest that pupae flakes are in accordance with the Indonesia National Standard for Cereal and are a good alternative as a functional food.

Keywords: Antibacterial, antioxidant, pupae flakes, pupae meal, Samia cynthia ricini

INTRODUCTION

Pupae is a transitional phase in the metamorphosis of a silkworm larva to a moth (imago) (Endrawati and Fuah, 2012). Silkworm pupae are a byproduct of silk fiber spinning that is not generally used and creates pupae accumulation, which is common in the silk fiber spinning industry. At the same time, the weight of pupae is nearly 80% of that of a whole cocoon. Pupae are also high in organic matter. For example, Eri silkworm pupae contain beneficial nutrients such as 54.6 ± 0.56% protein, 26.20 ± 0.35% fat, 3.80 ± 0.67% ash, 3.45 ± 0.06% crude fiber and 3.45 ± 0.06% carbohydrates (Longvah et al., 2011). Pupae can become an organic pollutant in the environment if improperly handled. Pupae is a functional food that contains bioactive compounds and high nutrition content, especially protein. Therefore, pupae can be beneficial for human health. The form of flour such as Bombyx mori pupae flour can lower blood glucose levels without causing long-term effects...
such as glycopenia. *Bombyx mori* pupae flour also contains active ingredients such as 1-deoxynojirimycin (DNJ) and polyhydroxy alkaloids, which help to lower blood glucose levels (Yang et al., 2009; Suk et al., 2016). Previous research has also found that *Eri* pupae flour has a low toxicity (LC50 value of 72.84 ppm). As a result, this pupae flour may be an excellent diabetic food option. Pupae are another insect product in the 1900 edible insects list (Jongema, 2012).

The high nutritional value contained in silkworm pupae can be used as an alternative food ingredient to produce flake pupae (Sinaga, 2017). Flakes are the food products that are usually consumed for breakfast. The use of *Eri* silkworm pupa flour as a substitution material for making flakes must meet the aspect of food safety. According to Rahayu and Nurwitri (2012), food problems in Indonesia are caused mainly by biological damage caused by contamination of pathogenic microbes that contaminate food products, which cause infections and health problems due to contamination by *E. coli* and *S. aureus* bacteria.

The production and analysis of flakes using the substitution of *Eri* silkworm pupa flour need to be carried out since the food product’s nutritional compositions and safety status are critical for human consumption. Therefore, this research aims to investigate the physicochemical properties and antioxidant activity of *Eri* silkworm pupae flake and its antibacterial properties.

**MATERIALS AND METHODS**

**Material preparation**

*Eri* silkworm pupae, harvested from the silkworm farm Kupu Sutera, were washed to remove mucus and dirt. The pupae were then steamed for 10 min before drying for 40 min in a Memmert UN 55 (Germany) oven at 100 °C. The pupae were then blended to make flour. Pupae flour was sieved with mesh and baked at 70 °C for 50 min.

**Making of pupae flake**

The formulation of pupae flakes follows Susi et al. (2019), which was modified by adding *Eri* silkworm pupae flour at a treatment percentage of 0%, 4% and 8%. The formulations of pupae flakes are shown in Table 1. The process begins with mixing ingredients such as tapioca flour, wheat flour, pupae flour, honey, margarine, vanilla and salt, stirring for 10 min and steaming for 60 min at 100 °C. Following that, grinding and the addition of water form a dough. The flakes dough formed was extruded three times, then flaking was processed two times. Finally, the formed samples were baked in an oven at 170 °C for 10 min. The following is the formulation for *Eri* silkworm pupae flakes.

**Physicochemical analysis**

Analysis of pH, moisture content, fat, ash and protein refer to the analysis of AOAC (2005), while the total carbohydrate content using the carbohydrate by difference method [100% - (water content + ash + protein + fat)]. The N-free protein content indicates the number of carbohydrates that can be digested from a food ingredient. The *A* (water activity) value is measured using an *A* meter Novasina MS1, as Salejda et al. (2014) described. Two g of the sample was mashed and then placed in the chamber's inner container.

**Antibacterial activity analysis**

*Total plate count (TPC)*

TPC was calculated using the SNI 2897:2008 method. A total of 10 g of pupae flour was aseptically placed in a sterile container. For a 10^1 dilution, 90 mL of 0.1% sterile buffered peptone water (BPW) was added and homogenized for 1 to 2 min. The sample was then diluted 10^2 with 9 mL BPW. This process is repeated until 10^6 is reached. Furthermore, 1 mL of each dilution was taken and placed in a Petri dish in a dupplo fashion. Following that, 15-20 mL of plate count agar (PCA) liquid medium cooled to 45 ± 1 °C is poured and homogenized in a dish, rotated to form a figure of eight, and allowed to stand until solid and then incubated at 34-36 °C for 24-48 h by placing the dish upside down. All growing colonies were counted as TPC by the Bacteriological Analytical Manual (BAM) method (FDA, 2001).

**Examination of Salmonella sp., *E. coli* and *S. aureus***

A total of 10 mL of pupae flour extract was reacted with

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**Table 1: Pupae flakes formulation.**

<table>
<thead>
<tr>
<th>Material</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polenta flour (crude corn flour) g</td>
<td>P1 (0%)</td>
</tr>
<tr>
<td></td>
<td>P2 (4%)</td>
</tr>
<tr>
<td></td>
<td>P3 (8%)</td>
</tr>
<tr>
<td>Pupae flour (g)</td>
<td>0</td>
</tr>
<tr>
<td>Margarine (g)</td>
<td>10</td>
</tr>
<tr>
<td>Water (mL)</td>
<td>50</td>
</tr>
<tr>
<td>Honey (mL)</td>
<td>10</td>
</tr>
<tr>
<td>Salt (g)</td>
<td>1.0</td>
</tr>
<tr>
<td>Vanilla extract powder (g)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

P1: 0% substitution of *Eri* silkworm pupae flour, P2: 4% substitution of *Eri* silkworm pupae flour and P3: 8% substitution of *Eri* silkworm pupae flour.
90 mL of 0.1% sterile buffered peptone water (BPW) in a sterile container in an aseptic manner. First, the solution was homogenized by vigorous shaking for a few seconds to obtain a 10⁻¹ dilution. Next, approximately 1 mL of solution at 10⁻¹ dilution was put into a test tube containing 9 mL of sterile BPW to obtain a 10⁻² dilution. Then repeat until 10⁻⁴. In addition, 1 mL of each dilution was poured onto Salmonella shigella agar (SSA) media and eosin methylene blue agar (EMBA) media for E. coli. In addition, 1 mL of each dilution was poured onto Salmonella shigella agar (SSA) media and eosin methylene blue agar (EMBA) media for E. coli. The mixture was then incubated at 35 °C for 24 h. Salmonella sp. (+) colonies are transparent with a black spot in the middle, whereas E. coli (+) colonies are metallic green with a black spot in the middle. In addition, bacterial colonies growing on SSA media were observed. To examine S. aureus, 1 mL of each dilution was put into a sterile Petri dish in a dipplo fashion. Then, in each dish, add 15 mL to 20 mL of buffered peptone agar (BPA) media that has been mixed with egg yolk tellurite emulsion (5 mL to 95 mL of BPA media). Each dilution yielded 0.1 mL of suspension, which was inoculated in a Petri dish containing solidified BPA media. The suspension was spread over the surface of the agar medium until it was adsorbed. The mixture was then incubated at 37 °C for 24 h. The Bacteriological Analytical Manual (BAM) method was used to count growing colonies (FDA, 2001).

Antioxidant analysis

DPPH inhibitory activity (Tangkanakul et al., 2009)

1 g of the sample was crushed and soaked in 2.5 mL of 100% methanol for 2 x 24 h. Then, drying and pressing were carried out to obtain liquid methanol extract of pupae flour. The pupae methanol extract was combined into a 10 mL measuring flask and adjusted with methanol solvent. The methanol extract was placed in a dark glass bottle, tightly closed, and stored in the freezer (−25 °C) until analysis. A total of 0.15 mL of methanol extract from pupae flour was reacted with a 0.1 mM DPPH solution (methanol solvent) of 0.9 mL in a vial. The solution was incubated in a water bath at 37 °C for 30 min. The absorbance was then measured using a UV-Vis spectrophotometer Gene Quant 1300 at 517 nm. The free radical scavenging activity of DPPH (2,2-Diphenyl-1-picrylhydrazyl) was expressed in % scavenging activity (% SA) with the following formula: % SA = (1 - Absorbance of sample/Absorbance standard × 100%).

Antioxidant capacity (Tangkanakul et al., 2009)

The % SA values were converted based on a standard curve. The standard curve was obtained by measuring the absorbance of the ascorbic acid reaction with DPPH, which was analyzed using a spectrophotometer at a wavelength of 517 nm. The concentration of an ascorbic acid solution prepared was 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg per 100 mL of distilled water. Antioxidant capacity is expressed as equivalent mg of vitamin C (VCE) per 100 g of pupae flour.

RESULTS AND DISCUSSION

Physicochemical properties of Eri silkworm pupae flour

The physicochemical properties of Eri pupae flour were conducted using two treatments, notably, 60 mesh and 80 mesh sieves. The flour of both treatments is seen in Figure 1. The analysis results for the physicochemical characteristics of Eri pupae flour are shown in Table 2.

pH value

Potential hydrogen (pH) is the amount of hydrogen ion concentration (H⁺) in a solution that indicates the solution's acidity and alkalinity (Ngafifuddin et al., 2017). The analysis of variance results revealed that particle size had no significant effect (P>0.05) on the pH value. The pH levels of the Eri silkworm pupae flour were 6.01-6.05 for P2 and 5.92-6.04 for P1. This value is within the range of cassava flour pH values of 5.51-5.60 (Sari et al., 2013), breadfruit flour 6.09, soy flour 6.67, corn flour 5.44, wheat flour 5.84 and tapioca flour 6.07 (Huang et al., 2019). The result shows that the pH value of Eri pupae flour meets flour quality standards based on the Indonesia National Standard for Flour (SNI 01-3751-2006), proving that it can be used and is safe as food.

Water activity (Aw)

Water activity can be used to predict the amount of available water and for biochemical processes such as mold, yeast and bacteria growth in materials. Water activity is measured in a range of 0.0 to 1.0. The statistical analysis revealed that particle size had no significant effect (P>0.05) on the Aw value of the Eri pupae flour. The Aw value of corn flour ranged between 0.23 and 0.32 (Fasoyiro et al., 2016). Mold grows best at Aw 0.60-0.70, yeast at 0.80-0.90 and bacteria at 0.90,
According to Winarno (2007). Furthermore, S.
aureus can grow with an Aw value of 0.86 (Jay et al., 2005). Based on this result, Aw Eri pupae flour is safe as food. This result is in line with microbiological analysis that showed negative in Salmonella sp., a low total plate count and low the present of S. aureus and E. coli bacteria.

Water content

The water content in food material is used to maintain the durability of food ingredients. A product’s resistance to microbe increases with lower water content, which should result in a longer expected shelf life (Sakti et al., 2016). The moisture content of Eri silkworm pupae flour ranges from 9.85% to 10.22%. According to SNI 01-3751-2006, the maximum water content standard for wheat flour is 14.5%, breadfruit flour has a water content of 12.6%, soy flour has a water content of 6.6%, corn flour has a water content of 11.1% and wheat flour has a water content of 12.2% (Huang et al., 2019). As a result, the water content of Eri pupa flour fulfills Indonesia National Standard (SNI 01-3751-2006).

Fat value

Fat content is crucial in emulsion products (Partogi et al., 2012). The statistical analysis results of pupae flour fat were not significantly different (P>0.05) when particle size differences were considered. Pupae flour particle size P2 (mesh 80) had a fat content of 7.84%, while P1 (mesh 60) had a fat content of 7.68%. The fat content has a significant impact on the material’s durability. If the fat content of the ingredients is high, fat oxidation will accelerate rancidity (Ketaren, 2005). Breadfruit flour has a fat content of 0.85%, soy flour has a fat content of 19.3%, corn flour has a fat content of 0.57%, wheat flour has a fat content of 1.51% (Huang et al., 2019) and Bombyx mori pupae flour has a fat content of 28.70% (Astuti and Kusharto, 2009).

Ash value

Ash remains an inorganic component after all the organic carbon has been burned (Haryasyah et al., 2009). The particle size analysis results did not differ significantly (P>0.05) from the ash content. P2 (mesh 80) pupae flour had a 6.76% ash content, while P1 (mesh 60) had a 6.93% ash content. SNI 01-3751-2006 specifies a maximum ash content of 0.7% for flour products. Bombyx mori pupae flour had an ash content of 3.50%, breadfruit flour 3.0%, soy flour 5.2%, corn flour 1.5% and wheat flour 1.1%. The high mineral content of Eri pupa flour is attributed to Eri pupa chitin (Haryasyah et al., 2009).

Protein value

Protein is an essential food nutrient for the body because it serves as a fuel, a building material and a regulator. Statistical analysis revealed that particle size was not significantly different (P>0.05) from protein content. Particle size P2 (mesh 80) protein content was 64.09%, while particle size P1 (mesh 60) protein content was 62.53%. According to SNI 01-3751-2006, the minimum protein content for flour products is 7.0%. Breadfruit flour has a protein content of 3.42%, soy flour has a protein content of 42.84%, corn flour has a protein content of 0.21% and wheat flour has a protein content of 12.74% (Huang et al., 2019). Bombyx mori pupae flour has a protein content of 37.32% (Astuti and Kusharto, 2009). The protein content of the Eri pupae flour is higher than that of other flour. Therefore, it is excellent as a protein source.

Crude fiber value

Statistical analysis revealed that the particle size did not differ significantly (P>0.05) from the crude fiber content of the pupae flour. The crude fiber content of the Eri pupae flour averaged 10.34% in P1 (60 mesh) and 7.95% in P2 (80 mesh). According to Huang et al. (2019), the crude fiber content of various flour products was 1.56% for breadfruit flour, 0.79% for soy flour, 1.10% for corn flour and 1.35% for wheat flour.

Carbohydrate value

The carbohydrate content of the Eri pupae flour was calculated using the formula (carbohydrate by difference). This method is less accurate because fiber (which does not produce energy in the body’s metabolism) is

Table 2: pH, Aw and proximate analysis of Eri silkworm (S. cynthia ricini) pupae flour.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.99 ± 0.07</td>
<td>6.03 ± 0.20</td>
</tr>
<tr>
<td>Aw</td>
<td>0.36 ± 0.00</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>9.85 ± 0.09</td>
<td>10.22 ± 0.04</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>7.68 ± 0.09</td>
<td>7.84 ± 0.22</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>6.76 ± 0.08</td>
<td>6.93 ± 0.01</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>62.53 ± 0.09</td>
<td>64.09 ± 0.28</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>10.34 ± 0.09</td>
<td>7.95 ± 0.52</td>
</tr>
<tr>
<td>Carbohydrate by difference (%)</td>
<td>13.19</td>
<td>10.95</td>
</tr>
</tbody>
</table>

P1: Pupae flour of Eri silkworm size 60 mesh; P2: Pupae flour of Eri silkworm size 80 mesh. The numbers in the same row that are not followed by letters are not significantly different at the 5% test level (P>0.05).
calculated as a percentage of carbohydrates. As a result, the results of product energy calculations are frequently overestimated (Winarno, 2007). Eri pupae flour had an average protein content of 13.18% at P1 (60 mesh) and 10.95% at P2 (80 mesh). The data were compared with the carbohydrate content of various flours, according to Huang et al. (2019), 80.1% breadfruit flour, 26.1% soy flour, 88.1% corn flour and 72.5% wheat flour.

### Antioxidant activity of pupae flour

The antioxidant activity and antioxidant capacity were determined in this study using DPPH (1,1-diphenyl-2-picrylhydrazil) inhibitory activity and antioxidant capacity. Antioxidant activity is calculated as the percentage of inhibition by comparing the absorbance of the sample to the standard. Antioxidant capacity refers to the number of antioxidants based on the ascorbic acid-DPPH inhibition calibration curve.

The statistical analysis revealed that the particle size difference in Eri silk pupae flour had no significant effect (P>0.05) on the value of DPPH inhibition and antioxidant capacity (Table 3). However, Eri silk pupae flour sieved through an 80 mesh (P2) sieve had a higher percentage of antioxidant activity and capacity than flour sieved through a 60 mesh (P1) sieve. Due to different particle sizes that affected the extraction process, there was a variation in the percentage of activity and antioxidant capacity between the two treatments (Sakalaty et al., 2021). According to Maulida and Guntari (2015), particle size influences the total anthocyanin content of black rice extract.

Antioxidant capacity values for Eri silk pupae flour using an 80 mesh (P2) sieve ranged from 202.64 to 246.35 mg EVC 100 g⁻¹ (Table 3). According to Tangkanakul (2009), the antioxidant capacity of ingredients is classified as very high (>500 mg VCE 100 g⁻¹), high (200-500 mg VCE 100 g⁻¹), medium (100-200 mg VCE 100 g⁻¹) and low (100-200 mg VCE 100 g⁻¹). The high antioxidant capacity in both treatments was due to the high phenolic content of Eri silk pupae (Deori et al., 2014). In addition, Eri silk pupae contain bioactive compounds, such as α-linolenic, arachidonic and lignoceric acids (Lokeshwari et al., 2019). Furthermore, the IC₅₀ value of Eri silk pupae is 68 µg/mL (68 mg/L), indicating that it is a potent antioxidant. A compound is said to be very strong if the IC₅₀ value is less than 50 mg/L, strong if the IC₅₀ value is between 50 and 100 mg/L, moderate if the IC₅₀ value is between 101 and 150 mg/L and weak if the IC₅₀ value is greater than 150 mg/L (Phongpaichit et al., 2007).

### Physicochemical properties of Eri silkworm pupae flake

The physicochemical characteristics of the flake Eri pupae were carried out in three treatments, namely 0%, 4% and 8% of the Eri silkworm pupae flour (S. cynthia ricini) substitution. The appearances of flake products are seen in Figure 2. Table 4 displays the test results for the physicochemical properties of the Eri pupae flake.

### pH value

The pH value of flake pupae ranged from 6.09 to 6.11 and did not differ significantly between treatments (P>0.05). These results are still considered acceptable in the food industry. Flakes and pasta have a neutral pH range of 5.0 to 6.0 and are neither acidic nor alkaline (Okafor and Usman, 2015). The presence of proteins, which are decomposed by proteolytic enzymes and bacteria into carboxylic acids, sulfide acids, ammonia and other acids, can cause the pH of food to change during storage (Rani et al., 2019).

### Water activity (Aw)

The Aw value of flake pupae ranged from 0.48 to 0.50 and did not differ significantly between treatments (P>0.05). Water activity can predict product safety and stability regarding microbial growth, chemical and biochemical reaction rates, and physical properties (Panja...
Fat oxidation is extremely slow at $A_w$ values of 0.2-0.3 (Bell, 2007). Most bacteria cannot grow at $A_w$ values less than 0.87. Some xerophilic fungi thrive at $A_w$ values ranging from 0.75-0.65. (Tapia et al., 2007). As a result, if the $A_w$ value is maintained, the quality of pupae flake can be protected from microorganisms and fat oxidation.

**Water content (%)**

The statistical analysis showed that various levels of pupa flour addition significantly affected the pupae flakes' water content ($P<0.05$). The water content of the P3 treatment (8%) differed significantly from that of the P1 (0%) and P2 (4%). As a result, the water content of the pupae flakes was found to be between 2.43% and 2.82%. According to SNI 01-4270-1996, the maximum flake water content was 3%. It was found that the lower the addition of pupae flour used, the results obtained would be lower the water content value. This result may be caused water content in pupa flour higher than crude corn flour.

**Ash value**

The addition of pupae flour to the ash content of pupae flake had a significant effect on all treatments ($P<0.05$). The ash content of the P3 treatment (8%) differed significantly from that of the P1 (0%) and P2 (4%). As a result, the water content of the pupae flakes was found to be between 2.43% and 2.82%. According to SNI 01-4270-1996, the maximum flake water content was 3%. It was found that the lower the addition of pupae flour used, the results obtained would be lower the water content value. This result may be caused water content in pupa flour higher than crude corn flour.

**Fat value**

The fat content of pupae flake ranged from 6.61% to 7.78% and significantly affected all treatments ($P<0.05$). Fat contributes calories while also improving the texture and taste of food. In addition, fat is a more efficient energy source for the body, with one gram producing 9 kcal of energy (Nurhidayanti et al., 2017). According to SNI 01-4270-1996, the quality requirement for flake fat content is a maximum of 7.0%.

**Protein value**

The difference in pupae flour concentration ($P<0.05$) affected the protein content of pupa flakes. The more pupae flour was added, the more the protein content in the product increased. The protein content of pupae flakes ranged between 6.24% and 8.23%. Protein is an essential food substance for the body because it serves as fuel and a building and regulatory substance (Widiatmoko and Estiasih, 2015). The quality requirements for flake protein content are at least 5.0%, according to SNI 01-4270-1996. This result showed that pupae flakes is a good source of protein.

**Carbohydrate value**

The carbohydrate content of pupa flakes was affected by the difference in pupae flour concentration ($P<0.05$). The addition of pupae flour reduced carbohydrate levels due to P1 utilizing more polenta flour than P2 and P3. Yellow corn flour has an 86.35% carbohydrate content (Lawalata et al., 2019). Moreover, both control and pupae flour addition met the quality requirements of SNI 01-4270-1996 for at least 60.0%.

**Antioxidant activity of pupae flake**

The antioxidant test on pupae flake was based on the best antioxidant value from pupa flour; thus, pupae flake with an 80 mesh size sieve was tested. Table 5 shows the results of the activity and antioxidant capacity analysis of Eri silkworm pupae flakes.

Statistical analysis showed that different levels of Eri silkworm (0%, 4% and 8%) had a significant effect on the activity and antioxidant capacity produced ($P<0.05$). The treatment of a 4% (P2) level of Eri silkworm pupae flour yielded the best value of activity and antioxidant capacity, with a percentage of antioxidant activity of 91.75 ± 0.67%.
and an antioxidant capacity of 253.86 ± 1.76 mg VCE/g. Following that, 8% (P3) pupa flour was administered, with an antioxidant activity percentage of 89.02 ± 3.04% and an antioxidant capacity of 246.73 mg VCE/g. Meanwhile, the 0% level (P1) had the lowest antioxidant activity and capacity, with 45.39 ± 5.75% antioxidant activity and 138.62 ± 1.98 mg VCE/g capacity. The differences in activity and antioxidant capacity in pupae flakes were due to the presence of flavonoids and phenolic acids in silkworm pupae, which can absorb, neutralize and reduce the activity of free radicals when pupae flour is substituted (Ghosh et al., 2020). Furthermore, a 0-dihydroxybenzene structure is associated with phenolic acids, which can enhance antioxidant activity (Singh et al., 2009). In addition, the IC50 value of 68 µg/mL for the Eri silkworm pupae, which was in the strong category, which have a good effect on antioxidant activity in the pupae flakes product (Lokeshwar et al., 2019).

**Antibacterial activity of pupae flour**

Table 6 shows the results of microbiological identification tests on Eri silkworm pupae flour sizes 60 mesh and 80 mesh. The Total Plate Count analysis results on samples of Eri silkworm pupa flour with 60 mesh and 80 mesh sizes met the SNI 3751: 2009 standard, i.e., a maximum of log 5 CFU/g. Salmonella sp. was not identified in Eri silk pupae flour, and the number of E. coli bacteria in both treatments was <10 APM/g.

The statistical analysis of the number of S. aureus found in Eri silk pupae flours with particle sizes of 60 mesh and 80 mesh resulted in no significant difference (P>0.05). The presence of S. aureus contamination in the sample could be attributed to the high protein content of Eri silk pupa flour, which accounts for approximately 54% of the dry weight (Longvah et al., 2011). Ekawati (2016) stated that S. aureus is a bacterium that thrives in foods high in protein, sugar and salt. S. aureus bacteria can contaminate food during handling and processing because they can spread through the skin, hair and respiratory tract. Furthermore, equipment and the environment can be sources of S. aureus pollution (SNI 7388-2009) (BSN, 2009). As a result, S. aureus contamination is most likely the result of contamination during the flour processing process, the tools used, the air, and the workers. One of the pathogens that cause foodborne illness is S. aureus bacteria. S. aureus bacteria are capable of producing toxins (enterotoxins) in food when the cell count is >106 colonies/g, where these toxins are stable and resistant at high temperatures and environmental conditions that experience freezing and drying (Yennie et al., 2022).

Table 5: Value of the activity and antioxidant capacity of Eri silk pupae flake (S. cynthia ricini).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antioxidant activity (%)</th>
<th>Antioxidant capacity (mg VCE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (0%)</td>
<td>45.40 ± 7.57a</td>
<td>138.62 ± 1.98b</td>
</tr>
<tr>
<td>P2 (4%)</td>
<td>91.75 ± 0.67a</td>
<td>253.87 ± 1.76a</td>
</tr>
<tr>
<td>P3 (8%)</td>
<td>89.02 ± 3.04a</td>
<td>246.73 ± 7.96a</td>
</tr>
</tbody>
</table>

P1: Substitution with Eri silk pupae flour 0%, P2: Substitution with Eri silk pupae flour 4% and P3: Substitution with Eri silk pupae flour 8%. The numbers in the same column followed by letters are significantly different at the 5% test level (P<0.05).

Table 6: Microbiological analysis of Eri silkworm (S. cynthia ricini) flour with different particle sizes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plate count (log CFU/g)</td>
<td>P1</td>
</tr>
<tr>
<td>Salmonella sp. (log CFU/g)</td>
<td>Negative</td>
</tr>
<tr>
<td>Escherichia coli (APM/g)</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Staphylococcus aureus (log CFU/g)</td>
<td>3.21 ± 0.61</td>
</tr>
</tbody>
</table>

P1: Eri silk pupae flour with 60 mesh size, P2: Eri silk pupae flour with 80 mesh size. The numbers in the same row that are not followed by letters are not significantly different at the 5% test level (P>0.05).
Table 7: Results of microbiological analysis of Eri silkworm pupae flakes (S. cynthia ricini).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
</tr>
<tr>
<td>Total plate count (log CFU/g)</td>
<td>4.08 ± 1.04</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Negative</td>
</tr>
<tr>
<td>Staphylococcus aureus (log CFU/g)</td>
<td>3.02 ± 0.14</td>
</tr>
</tbody>
</table>

P1: Substitution with Eri silk pupae flour 0%, P2: Substitution with Eri silk pupae flour 4% and P3: Substitution with Eri silk pupae flour 8%. The numbers in the same row that are not followed by letters are not significantly different at the 5% test level (P>0.05).

CONCLUSION

The particle size of pupae flour did not affect its physicochemical properties or antioxidant activity and microbiologically, it met the Indonesia National Standard for flour. Moreover, the flakes product with pupae flour substitution influenced its physicochemical properties, with the best level at 8% pupae flour for the highest protein content and increased the flakes product's activity and antioxidant capacity. Based on the microbiological analysis of pupae flakes products, the substitution of Eri pupa flour was still within safe limits for microbial contamination in the total plate count (TPC) test, identification of E. coli and S. aureus bacteria so that it is safe for human consumption. Therefore, pupae flakes are in accordance with the Indonesia National Standard for Cereal and are a good alternative as a functional food.

ACKNOWLEDGEMENTS

The research presented in this publication was financially supported by IPB University through Dosen Muda Research Program. Authors would like to thank all the research team members.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES


Jongema, Y. (2012). List of edible insect species of the world. Laboratory of Entomology, Wageningen University, Wageningen.


