Improvement of Bread Nutrition with The Addition of Coffee Silverskin as a Source Of Dietary Fiber And Antioxidants

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ABSTRACT

Obesity can be prevented by reducing the intake of high carbohydrates and fats, then replacing with high dietary fiber. Coffee silverskin is a by-product of coffee beans with high dietary fiber (54-74%) and antioxidants, which can potentially treat obesity. Indonesia has a traditional bread named "Gandjel Rel" from Semarang, which has a box-shaped, brown color with a sprinkling of sesame on the surface, and cinnamon flavor. The aims of this study are to increase its functional properties. In the bread making process, silverskin from Robusta coffee (Coffea canephora) flour is added with a variation of 0; 1; 2.5; and 5% (dw). The dough was baking at a temperature of 120°C for 30 minutes. The bread was analyzed in terms of its dietary fiber, antioxidant activity by DPPH method, physical (hardness and color intensity), chemical properties (proximate), and sensory analysis. Bread with robusta coffee silverskin has higher dietary fiber (3.33-7.18% (w/w) insoluble fiber and 0.25-0.77% (w/w) soluble fiber) and antioxidant activity (26.23-31.36 g trolox eq./ 100 gr). Additional of coffee silverskin in the bread dough did not change the texture significantly, however, it did alter its physical appearance due to coffee silverskin’s brown color obtained from the coffee bean roasting process, so reducing brightness level and the yellowness of the bread. The panelists preferred bread by adding 1% robusta coffee silverskin. This bread has higher protein (11.33%, w/w), than conventional bread’s protein (8%). In addition, this bread contains dietary fiber and antioxidants, so it has the potential as a functional food.

Keywords: Antioxidant, Bread, Dietary Fiber, Obesity, Robusta Coffee Silverskin

INTRODUCTION

Based on the 2018 Basic Health Research, the prevalence of overweight in children aged 5-12 years and adults aged > 18 years was 18.8% and 13.5%, respectively. Meanwhile, the prevalence of obesity in children aged 5-12 years and adults aged > 18, respectively, amounted to 10.8 and 28.7%. Based on WHO guidelines for Asian adults, the category is overweight if BMI is between 23–24.9 kg/m², and the obese category is if BMI is ≥ 25 kg/m². Body mass index (BMI) is determined by comparing body weight and height. One of the causes of overweight and obesity is the accumulation of body fat due to energy intake exceed the energy used (Saraswati et al., 2021). Sedentary behavior, lower physical activity,
and higher intake of fried foods, sweet foods and beverages, also food high in refined carbohydrates and fatty also significant contributors to overweight and obesity in general (Nurwanti et al., 2018).

Prevention of obesity could be done by reducing the intake of high in carbohydrates and fats, then replacing them with foods high in fiber. Dietary fiber consumption is reported to be able to reduce the risk of obesity because of its role in regulating energy balance and increasing satiety. Dietary fiber reduces food energy density or calories, so consuming it has less energy than other foods in the same portion. High dietary fiber can reduce digested energy intake to reduce the risk of obesity (Waddel and Orfila, 2022).

Coffee consumption in Indonesia increased by 8.22% per year, indirectly increasing the number of coffee by-products. Dry, semi-dry or wet coffee processing produces by-products reaching 45-50% of the whole coffee fruit, namely skin, husk, pulp, parchment, and silverskin (Santos et al., 2021). Coffee silverskin is a by-product obtained from the roasting process, which has a thin layer like the epidermis attached to coffee beans.

The use of coffee silverskin is still limited to making compost and biogas (Santos et al., 2021). Coffee silverskin is rich in nutrients, such as dietary fiber (60%) and minerals (8%), which are dominated by potassium, magnesium and, calcium, and protein (20%) (Santos et al., 2021). In addition, several bioactive compounds in coffee silverskin have anti-obesity properties, namely caffeine (1.37 g/100 g) and polyphenols such as chlorogenic acid (56.5 mg CGA eq./g) and 5-cafeoylquinic acid (21.3 mg/g) (Martinez-Saez et al., 2014). Therefore, coffee silverskin can potentially be processed and utilized as a functional food for anti-obesity. Coffee silverskin has been used in food products, such as beverages, yogurt, bread, cookies, biscuits, and cakes (Klingel et al., 2020; Martinez-Saez and del Castillo, 2018; Bertolino et al., 2019; Pourfarzad et al., 2013; Gocmen et al., 2019; Ateş and Elmacı, 2018; 2019).

One of Indonesian’s traditional breads, named "Gandjel Rel" bread from Semarang, which has a box-shaped, brown color with a sprinkling of sesame on the surface and a cinnamon flavor. The brown color is obtained using palm sugar with a low glycemic index (35.56) (Ismail et al., 2020). Consumption of foods with a low glycemic index can improve insulin sensitivity, delay hunger, and reduce the rate of glucose absorption and it is useful in treating and preventing obesity (Pereira et al., 2014; Schwingshackl and Hoffman, 2013). The purpose of this study was to increase the functional properties of "Gandjel Rel" bread through the addition of robusta coffee silverskin in the bread dough in order to enrich the content of dietary fiber and antioxidant activity by DPPH method so that it is hoped that it can be used to treat obesity.

MATERIALS AND METHODS
Tools and Materials
The tools in this study were analytical balance (Excellent HZK, Indonesia), dehydrator (16 Tray FDH-16 Wirastar, Indonesia), a grinder (4MFJ - Huang Cheng-800g, China), texture analyzer (TVT-300XP, Sweden), chromameter (Konica Minolta CR-400, Japan), oven (Mito Fantasy Mo-888, Indonesia), and spectrophotometer (Genesys 150 UV-Vis, USA).

The main ingredient in this study was coffee silverskin from Robusta coffee obtained from coffee farmers in Ungaran, Central Java, Indonesia. The ingredients used in making the bread were wheat flour, bread flour, palm sugar, egg yolks, margarine, full cream milk, baking soda, baking powder, cinnamon powder, vanilla, salt, and white sesame obtained from a shop in Semarang.
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Coffee Silverskin Extraction

Coffee silverskin extraction was carried out based on patent WO 2013/004873 (del Castillo, 2013) with several modifications in dried process. A total of 50 g of silverskin was extracted with 1 liter of boiling water for 10 minutes. The extract was filtered and dried using a Food Dehydrator (16 Tray FDH-16 Wirastar, Indonesia) at 50˚C until a constant weight was obtained. The dried extracts were ground using a grinder (4MFJ - Huang Cheng - 800g, China) to form flour. The resulting silverskin (S) extract flour is filtered using a sieve with a size of 30 mesh and stored in a closed container.

Bread Making

The bread making is based on the recipe for making “Gandjel Rel” bread which was obtained from a native person from Semarang with some modifications. The first step is to weigh the wheat flour with the addition of robusta coffee silverskin flour with different concentrations (0%, 1%, 2.5%, and 5%) with a total weight of 50 g. The composition of other ingredients is made the same (Table 1), such as bread flour, egg yolks, margarine, full cream milk, baking soda, baking powder, cinnamon powder, and vanilla. All ingredients are mixed until everything is evenly mixed. Palm sugar that has been dissolved is added to the dough. Leave the bread dough for 30 minutes. The dough is molded in rectangular molds measuring 5 x 2 cm with a thickness of ±0.5 cm. After that, the dough was put into the oven (Mito Fantasy Mo-888, Indonesia) at a temperature of 120˚C for 30 minutes and then cooled to room temperature (±32˚C).

Research design

This study used an experimental method with a completely randomized design (CRD) with a single factor, namely the addition of robusta coffee silverskin flour with different concentrations (0%, 1%, 2.5%, and 5%) (dw). The experiment was carried out with 3 repetitions. Each analysis was performed 2 times a repetition.

Dietary Fiber Analysis

Insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and total dietary fiber (TDF) were determined using a gravimetric test based on the AOAC-991.43 and AACC-32.07.01 methods (McCleary et al., 2012). Dry samples were gelatinized with α-amylase enzymes (Sigma-Aldrich Ireland Ltd). After gelatinization, the samples were digested with protease (Sigma-Aldrich Ireland Ltd) and amyloglucosidase (Sigma-Aldrich Ireland Ltd) to remove protein and starch from the samples. The IDF is filtered and washed. The filtrate and washing water were combined with four volumes of 95% ethanol (Merck, Germany) at 60˚C to precipitate the SDF. The mixture is filtered, and the residue (soluble fiber) is dried and weighed. The total value of dietary fiber is calculated as the sum of insoluble dietary fiber and soluble dietary fiber. The analysis was repeated twice for each sample, and the results were expressed as a percentage by weight (%).

Antioxidant Activity Analysis

Determination of antioxidant activity was carried out based on the DPPH method (Molyneux, 2004). Bread samples were crushed using a porcelain cup, then added methanol solution (Merck, Germany) and proceeded with filtering. Samples were made at a concentration of 1,000 ppm in methanol solution, then diluted to obtain 200, 400, 600, 800, and 1,000 ppm concentrations. Each 4 mL was reacted with 1 mL of 0.2 mM DPPH solution (Sigma-Aldrich Ireland Ltd). Sample absorbance was measured using a spectrophotometer (Genesys 150 UV-Vis, USA) at a wavelength of 517 nm. A calibration curve was prepared using trolox.
as standard in the range of 5.62 to 75.87 mg/L ($r = 0.9896$). The results were expressed as g of trolox equivalent (TE)/100 g of dry weight.

**Physical Properties Analysis**

Analysis of physical properties includes the level of hardness and color intensity. The hardness level of the bread was measured using a texture analyzer (TVT-300XP, Sweden). Color intensity analysis was measured using a Chromameter (Konica Minolta CR-400, Japan), which is expressed as brightness values, L* (black-and-white), a* (green-red), and b* (blue-yellow).

**Sensory Analysis (Damat et al., 2019)**

Sensory analysis was carried out to determine the acceptability of the bread. A total of 20 untrained panelists were randomly selected to assess the acceptability of the 4 bread formulas using a structured quantitative acceptance assessment test. Each panelist was given a drink after assessing each sample to eliminate taste disturbances. The parameters assessed were texture, taste, aroma, and level of preference with a rating scale, namely dislike very much (1), dislike (2), like (3) and like very much (4).

**Chemical Properties Analysis**

Analysis of chemical properties included moisture, ash, carbohydrates, fats, and proteins according to standard AOAC procedures (2016). Each analysis was performed 2 times a repetition.

**Data analysis**

The data (dietary fiber, antioxidant activity, physical properties, and sensory) obtained was tested statistically with Analysis of Variance (ANOVA) analysis using IBM SPSS Statistics 23 (2015, United States) and continued with the Duncan's Multiple Range Test (DMRT) with a significance level of 95%. The data of chemical properties was tested statistically with T test.

**RESULTS AND DISCUSSION**

**Total Dietary Fiber and Antioxidants**

The total dietary fiber content in bread increased with robusta coffee silverskin flour concentration (Table 2). It was dominated by insoluble fiber content than the soluble fiber content in bread in all treatments. Coffee silverskin contains total dietary fiber ranging from 60-70%, most of which is insoluble fiber (53-64%), while soluble fiber is 7.6-8.8% (Pourfarzad et al., 2013; Behrouzian et al., 2016). Therefore, bread with robusta coffee silverskin flour at all concentrations contained more insoluble fiber than soluble fiber.

The addition of coffee silverskin to the production of Barbari bread, Iranian flatbread and gluten-free bread showed similar results in this study. Barbari bread and gluten-free bread with the addition of coffee silverskin had higher total dietary fiber with higher insoluble and soluble dietary fiber content compared to bread without the addition of coffee silverskin (Pourfarzad et al., 2013; Guglielmetti et al., 2019). These results indicate that coffee silverskin can be used for nutrification of dietary fiber in bread making.

Consumption of dietary fiber has a positive impact on the physiological functions of the body related to the management of obesity, which can occur through a direct mechanism by affecting intestinal function in the process of digestion, absorption, and appetite or through an indirect mechanism by affecting the composition and metabolism of the microbiota in the intestine (Waddell and Orfila, 2022). High-fiber foods can reduce calorie intake. Low-calorie intake is known to help the body regulate energy balance for weight maintenance (Church and Martin, 2017). Consumption of dietary fiber can also
trigger a satiety effect by stimulating the release of hormones such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1). The CCK hormone regulates satiety and the absorption and metabolism of nutrients (Burton-Freeman et al, 2002) while GLP-1 controls insulin and glucagon secretion, which is related to response and blood glucose levels (Ye et al, 2015).

The antioxidant activity of bread increased with the increased concentration of robusta coffee silverskin flour added (Table 2). Research by Guglielmetti et al. (2019) showed that adding coffee silverskin to gluten-free bread had an antioxidant activity that was 3.78 times higher when compared to control. Coffee silverskin has a high antioxidant component (169.5 mg/g) consisting of caffeine (53.3 mg/g), chlorogenic acid (56.5 mg/g), and flavonoids (6.3%), which contribute to its high antioxidant activity. (Iriondo-DeHond et al., 2017).

Gocmen et al. (2019) also reported that all cookies with coffee silverskin had significantly higher antioxidant capacities than control. The antioxidant properties of coffee silverskin are related to the content of dietary fiber in it, which is categorized as antioxidant dietary fiber (AODF). The dietary fiber of coffee silverskin has the highest antioxidant activity compared to the dietary fiber of other coffee by-products. The antioxidant activity of dietary fiber in silverskin is due to the binding of chlorogenic acid or other antioxidant compounds with fiber as a macromolecular matrix to form a complex carbohydrate structure (Quiros-Sauceda et al., 2015).

Martinez-Saez’s research (2014) showed that antioxidant drinks made from coffee silverskin Arabica and Robusta could reduce body fat in experimental animals by up to 30% and 29%. Total cholesterol and blood plasma triglyceride levels also decreased after 45 days of treatment, followed by a decrease in lipase activity as much as in vitro at a 36 mg/mL concentration. This shows that processed food from coffee silverskin can improve the functional properties of food products with the presence of antioxidant activity and dietary fiber content, which has the potential to be used as an anti-obesity agent.

**Bread Physical Properties**

The addition of robusta coffee silverskin flour did not affect the hardness of the bread because the addition was not too much (1-5%) (Table 3). Robusta coffee silverskin has a low water content of 4-7% (Ates and Elmacı, 2019) because it is processed through roasting, which can reduce its moisture, so it does not affect the hardness of the bread. This also happened to cookies that were added coffee silverskin (0; 2.5; and 7.5%) which had no different level of hardness (Gocmen, 2019). This shows that robusta coffee silverskin can be used as a food additive such as bread products, cakes, and cookies.

The brightness level of the four bread formulas (Table 3) shows that the more robusta coffee silverskin flour added, the lower the brightness of the bread. Robusta coffee silverskin has a dark brown color (Figure 1) obtained from the roasting process, which reduces the bread's brightness level. Adding coffee silverskin flour also reduced the yellowness of the bread, but not the redness. The addition of robusta coffee silverskin resulted in the bread being darker and yellower. Cake formulations with the addition of coffee silverskin also show a decrease in brightness and yellowness, resulting in cake being darker and yellower with an increase of coffee silverskin added. (Ates and Elmacı, 2018).

**Sensory Bread**

The addition of robusta coffee silverskin flour (1-5%) did not make a
difference in the panelists' assessment of the texture of the bread. Robusta coffee silverskin has a low water content of 4-7% (Ates and Elmacı 2019), because it is processed through roasting, which can reduce its moisture, so it doesn't change the texture of the bread.

The panelist's assessment of aroma and taste showed a scale of dislike as the concentration of robusta coffee silverskin added to the bread increased. Robusta coffee silverskin has a roasting aroma produced from the roasting process of coffee beans, so the more robusta coffee silverskin added, the stronger the aroma will be. Adding silverskin flour with a concentration of > 1% gives a bitter taste from the roasting process. Robusta coffee silverskin has a high caffeine and chlorogenic acid content, 53.3 mg/g and 56.5 (Iriondo-DeHond et al., 2019). Caffeine and chlorogenic acid result in a bitter taste (Santosa et al., 2020), so give a bitter taste in bread with more coffee silverskin.

The panelist's assessment based on the preference level showed that they liked all four samples. However, based on the aroma and taste assessment, they preferred the S1 formula, namely bread with the addition of 1% robusta coffee silverskin flour. Therefore, the best bread of the four formulas is bread with the addition of 1% robusta coffee silverskin flour.

**Bread Chemical Properties**

Bread with the addition of 1% robusta coffee silverskin, which is the best assessment of sensory analysis, was analyzed for its chemical composition. Adding robusta coffee silverskin flour increased the bread's protein and carbohydrate content (Table 5). Bread with robusta coffee silverskin has a higher protein (11-19%) and carbohydrate content (62-67%) than bread without adding coffee silverskin (Gemechu, 2020). Robusta coffee silverskin's water and fat content are low, as much as 4-7% and 2.6-5.2 (Gemechu, 2020), so it does not significantly affect bread.

**CONCLUSION**

Adding robusta coffee silverskin to bread can increase insoluble dietary fiber from 3.33-7.18% and soluble dietary fiber 0.25-0.77%. Robusta coffee silverskin also increases bread's antioxidant activity, which can increase functional properties that have the potential as an anti-obesity agent. Robusta coffee silverskin has a high antioxidant component (169.5 mg/g) consisting of caffeine (53.3 mg/g), chlorogenic acid (56.5 mg/g), and flavonoids (6.3%), which contribute to its high antioxidant activity of coffee silverskin. Robusta coffee silverskin did not affect the hardness of the bread but made it darker and yellower. But, panelists preferred bread with the addition of 1% robusta coffee silverskin (S1) with chemical properties of 11.33% protein, 17.40% fat, and 66.42% carbohydrates. Therefore, further research should improve the formulation by adding more robusta coffee silverskin flour because it can increase the content of dietary fiber and antioxidants activity.

**ACKNOWLEDGEMENT**

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Masyarakat Indonesia 20: 70–74. https://doi.org/10.14710/mkmi.20.1.70-74


Table 1. Bread Formulation Composition

<table>
<thead>
<tr>
<th>Material (gram)</th>
<th>S0</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parchment Flour</td>
<td>-</td>
<td>1</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>Wheat Flour</td>
<td>50</td>
<td>49</td>
<td>47.5</td>
<td>45</td>
</tr>
<tr>
<td>Bread Flour</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Full Cream Milk</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Baking Soda</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Baking Powder</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Vanila</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Egg Yolk</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cinnamon Powder</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Margarine</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Palm Sugar</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

Noted: S0 = without the addition of silverskin flour, S1 = the addition of 1% parchment flour, S2 = the addition of 2.5% silverskin flour, and S3 = the addition of 5% silverskin flour.

Table 2. Total Dietary Fibre (% w/w) and Antioxidants Activity (DPPH methods) of Breads (g Trolox eq./100 g)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insoluble Diet Fiber</th>
<th>Soluble Diet Fiber</th>
<th>Total Diet Fiber</th>
<th>Antioxidant Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>3.83±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.10±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.23±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>S1</td>
<td>3.33±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.52±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.23±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S2</td>
<td>4.37±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.29±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.66±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.11±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>S3</td>
<td>7.18±0.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.77±0.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.95±0.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>31.36±0.23&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Noted: All data is the average of 2 repetitions. The different letters in the same column show a significant difference with p<0.05.

Figure 1. A = Coffee Silverskin, B = Silverskin Flour, and C = Bread with adding of Silverskin

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Table 3. Hardness Level using texture Analyzer and Color Intensity Bread using Chromameter

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hardness Level</th>
<th>Color Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brightness Level (L)</td>
<td>Redness Level (a)</td>
</tr>
<tr>
<td>S0</td>
<td>56.92±7.04(^a)</td>
<td>46.70±0.04(^d)</td>
</tr>
<tr>
<td>S1</td>
<td>51.96±1.67(^a)</td>
<td>38.38±0.94(^c)</td>
</tr>
<tr>
<td>S2</td>
<td>51.68±3.16(^a)</td>
<td>35.47±0.27(^b)</td>
</tr>
<tr>
<td>S3</td>
<td>55.46±2.50(^a)</td>
<td>28.00±0.77(^a)</td>
</tr>
</tbody>
</table>

Noted: All data is the average of 2 repetitions. The different letters in the same column show a significant difference with p<0.05.

Table 4. Sensory Evaluation of Breads Formula (Damat, et al., 2019)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flavor</td>
</tr>
<tr>
<td>S0</td>
<td>3.50(^b)</td>
</tr>
<tr>
<td>S1</td>
<td>3.65(^b)</td>
</tr>
<tr>
<td>S2</td>
<td>3.43(^ab)</td>
</tr>
<tr>
<td>S3</td>
<td>2.25(^a)</td>
</tr>
</tbody>
</table>

Noted: All data is the average of 2 repetitions. The different letters in the same column show a significant difference with p<0.05.

Table 5. Bread Chemical Properties (% w/w) (AOAC Procedure)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Lipid (%)</th>
<th>Carbohydrate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>7.36±0.10(^a)</td>
<td>1.58±0.10(^a)</td>
<td>7.42±0.14(^a)</td>
<td>16.86±0.09(^a)</td>
<td>46.77±0.15(^a)</td>
</tr>
<tr>
<td>S1</td>
<td>6.02±0.16(^a)</td>
<td>1.82±0.04(^a)</td>
<td>11.33±0.18(^b)</td>
<td>17.40±0.04(^a)</td>
<td>66.42±0.10(^b)</td>
</tr>
</tbody>
</table>

Noted: All data is the average of 2 repetitions. The different letters in the same column show a significant difference with p<0.05.