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# Stability of pigments from *Termitomyces* sp. (termite mushroom), *Pleurotus citrinopileatus* (yellow oyster mushroom) and *Pleurotus djamor* (pink oyster mushroom) as natural food colouring

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## Abstract

Food colouring has been used in food items for a long time to enhance the natural colour of food, maintain colour consistency and enhance product attractiveness. The most popular food colouring is synthetic since it is more stable and less expensive. However, there is growing concern about how artificial food colouring affects human health. As a result, research into using natural food colouring as an alternative to synthetic colouring has grown. Due to the complexity of food products, natural food colouring is unstable and prone to degradation. Therefore, the goal of this study was to use the CIELAB colorimeter to ascertain the colour stability of the pigments found in *Termitomyces* sp. (termite mushroom), *Pleurotus citrinopileatus* (yellow oyster mushroom) and *Pleurotus djamor* (pink oyster mushroom). At three pH levels (3.0, 7.0 and 15.0), the stability of pigments derived from these mushrooms was assessed; the pigments were most stable at pH 7.0. Additionally, the pigments were heated from 40 to 100°C. The outcomes demonstrated that *P. citrinopileatus*'s yellow pigment was unstable at 40°C, 60°C 80°C and 100°C. When heated between 40°C and 80°C, the pigments derived from *P. djamor* and *Termitomyces* sp. remained stable. Ascorbic acid, citric acid, and sodium metabisulphite treatments had no discernible effects on the stability or colour of the pink pigment (a* values) as compared to the control.

## 1. Introduction

Synthetic food colourings can be a health hazard when added to food products. The use of synthetic colourant in foods has been reported to have effects on allergic reactions, carcinogenicity, hyperactivity in children, as well as underdevelopment of organ and cellular defence system of the mother and foetus (Galaffu *et al.*, 2015; Bora *et al.*, 2019; Reza *et al.*, 2019; Wu *et al.*, 2020). Azo colourants are widely produced and used as synthetic colouring agents in the food industry because of easy availability, high purity, and colour intensity, and low sensitivity to heat, light and chemical interactions. The azo linkage (N=N) exists in the chemical structure of every azo dye. This linkage is susceptible to breakage when exposed to chemical compounds such as heavy metals that enable conversion of this synthetic dye into its free form in the colon that makes it carcinogenic. In addition, the metabolic cleavage of the azo bond may also increase the production of oxidative stress in humans (Sarıkaya *et al.*,

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2012; Diacu, 2016). This causes overproduction of reactive oxygen species that could lead to numerous diseases such as cancer, cardiovascular diseases, a decline of the immune system and dysfunction of the brain and liver (Reis *et al.*, 2012; Sánchez, 2017; Lu *et al.*, 2021).

The use of synthetic dyes can have adverse health effects. A study by Çolakoğlu and Selçuk (2020) reported that the usage of an artificial food colourant known as Sunset Yellow during pregnancy may cause underdevelopment of organ and cellular defence system of the mother and foetus. Similarly, carmoisine food colourant was found to decrease the effectiveness of food absorption capacity of the intestinal surface of the animal because of a lower intestinal bacteria population, which causes growth retardation (Reza *et al.*, 2019). Hence, many companies are now interested in finding alternative sources of natural food colourants. Industrial applications of natural colouring in foods depend on stability and solubility of the natural pigment. The colourant should be stable to pH, heat, light, and antioxidants over the required shelf life of foods. The loss of colour is an indicator of poor product quality or a sign of deterioration.

The vibrant colours of mushrooms make it a suitable source of natural food colourant (Ahmad *et al.*, 2022). Termite mushroom (*Termitomyces* sp.) is a wild edible mushroom belonging to the Basidiomycetes fungi species (Figure 1a). This seasonal mushroom is the world's largest edible mushroom and has recently been investigated as alternative protein source (Morrison, 2022). Meanwhile, yellow oyster mushroom (*P. citrinopileatus*) is another Basidiomycetes fungi from the *Pleurotus* spp. that has a distinctive yellowish to golden upside cap colour. Although the upper cap is yellow in colour, the flesh of *P. citrinopileatus* is pure white with a soft texture as shown in Figure 1b. The cap is usually funnel-shaped with an average diameter of around 3.0 to 7.5 cm, while the white cylindrical stem is usually 3.0 to 6.0 cm long with a thickness of 0.3 to 1.2 cm. Pink oyster mushroom (*P. djamor*) is an edible mushroom with a bright pink colour as shown in Figure 1c. *P. djamor* is easy to cultivate in tropical countries such as Malaysia, Indonesia, Philippines, and Mexico because of its optimum growth under humid conditions of more than 80% and warmer temperature ranging between 26°C to 35°C (Zurbano *et al.*, 2017; Raman *et al.*, 2020).

Basidiomycetous fungi (mushrooms) can produce a wide range of colours and may potentially be a natural source of food colourant. Zhang *et al.* (2022) demonstrated the presence of melanin pigment in the caps of *P. citrinopileatus*, and *P. djamor*, where each pigment extract exhibited yellowish-brown and reddish-brown colour, respectively. With their vibrant yellow and pink colours, these mushrooms could potentially be used to produce natural food colourant. The morphology of melanin pigments extracted from *P. citrinopileatus*, and *P. djamor* exists as almost spherical amorphous solids with a unit's diameter of approximately 30-50 nm. Melanin can be found in abundance in the cell wall of coloured mushroom caps and forms cross-linking between pigments and polysaccharides (Camacho *et al.*, 2019; Zhang *et al.*, 2022).

The major problem in producing colourant from natural sources especially plants is the stability of their pigments against environmental factors and processing parameters such as pH, temperature, light, and oxidation that could potentially restrict their utilisation as commercial pigments for industries. It is important to point out that different pigment possesses different stability for these parameters (Poorniammal and Gunasekaran, 2015; Leong *et al.*, 2018). According to Ananga *et al.* (2013), the changes in anthocyanin pigment colours at different pH values are due to changes in the molecular species in the structure. Melanins have low solubility in water and organic solvents, partial solubility in concentrated sulphuric acid and nitric acid, and are fully soluble in sodium hydroxide (Glagoleva *et al.*, 2020). It is highly crucial for the pigments to be stable in antioxidants (usually available in

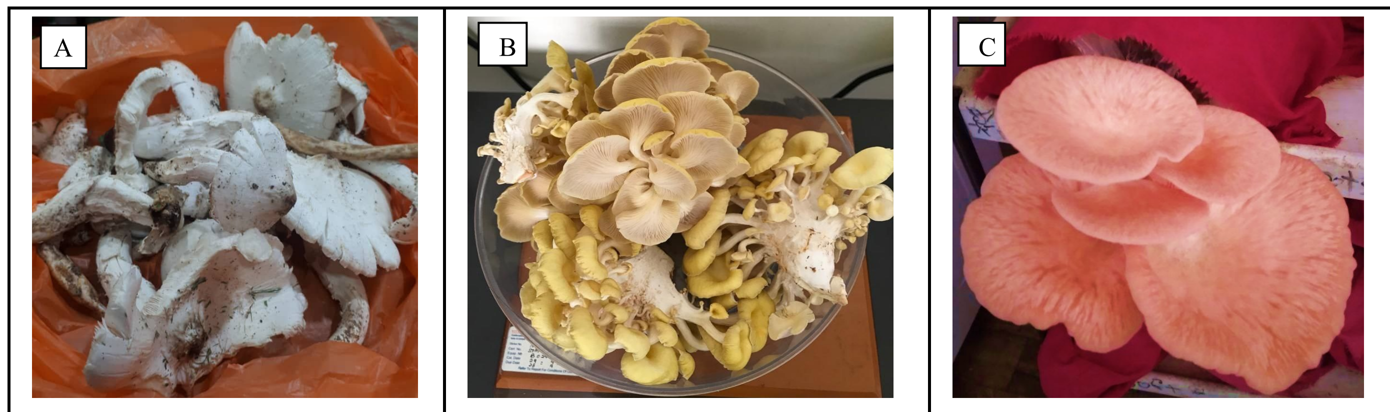


Figure 1. Images of (a) termite mushroom (*Termitomyces* sp.), (b) yellow oyster (*P. citrinopileatus)* and (c) pink oyster (*P. djamor).*

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food) and do not cause either pigment deterioration or antioxidant degradation (Li *et al*., 2023). Therefore, due to limited studies on the stability of these mushrooms pigment, hence, this research focused on determining the stability of pigments extracted from *Termitomyces* sp., (termite mushroom), *Pleurotus citrinopileatus* (yellow oyster mushroom), and *Pleurotus djamor* (pink oyster mushroom) in terms of pH, heat, light and antioxidants usually used in food industry.

## 2. Materials and methods

### *2.1 Raw materials*

Mushroom samples (*P. citrinopileatus* and *P. djamor*) were supplied by Nature Pick Sdn Bhd, which is located at Pusat Pembangunan Komoditi, Kompleks Jabatan Pertanian Serdang, 43400 Serdang, Selangor. The *Termitomyces* sp. was picked by hand from Kajang, Selangor. The mushrooms were packed in an icebox during transportation and once obtained were immediately cleaned to remove soil and dirt using soft tissue paper without washing. The mushrooms were then packed in a plastic wrapper and stored at -20°C in the freezer before the extraction of pigments.

### *2.2 Chemicals*

Methanol, ethanol, 1 mol/L NaOH, 1.5 mol/L NaOH, 2 mol/L NaOH, 0.1 N NaOH, 40% NaOH, 1 mol/L HCl, 2 mol/L HCl, 7 mol/l HCl, 0.1 N HCl, citric acid, sodium metabisulphite, ascorbic acid, butylated hydroxyl anisole. All chemicals used in this study was analytical grade and obtained from LabChem Sdn Bhd, Malaysia.

### *2.3 Pigment isolation*

The isolation and purification method of mushroom pigment was conducted following the method used by Zhang *et al*. (2022). The cap of the mushroom fruiting body was homogenised by blending it with solvent as described. The homogenised sample (10 g) was blended with 1.5 mol/L NaOH (300 mL) and then sonicated for 80 min. The suspension was subjected to centrifugation at 3500 rpm for 45 min. The supernatant was collected and transferred into the Erlenmeyer flask. The supernatant pH was adjusted to 1.5 using 7 mol/L HCl solution. The supernatant was precipitated at room temperature for 3 h and then centrifuged at 3500 rpm for 45 min. The precipitate was washed with deionized water until neutral pH was obtained. The precipitate was then dehydrated using a freeze dryer.

### *2.4 Pigment purification*

Pigment purification was carried out using with 10 mL of ethanol, chloroform, and ethyl acetate to remove sugar, proteins and lipids. 1 g of crude pigment was immersed in 7 mol/L HCl solution at 100°C for 2 h. The mixture was centrifuged at 3500 rpm for 45 min and the precipitate collected. The precipitate was washed three times with distilled water followed by ethanol, chloroform, and ethyl acetate for pigment purification and the insoluble pigment was left to dry at room temperature. The dried pigments were dissolved in 2 mol/L NaOH solution and the mixture centrifuged at 3500 rpm for 45 mins. The supernatant was collected and 2 mol/L HCl was added until the solution reached pH 1.5 and precipitated again. The precipitate was collected by centrifugation and washed with distilled water. The precipitation step was repeated four times. Finally, the precipitates were washed using distilled water until neutral pH was achieved and the pure pigments dried at room temperature (Zhang *et al*., 2022).

### *2.5 Pigment stability*

Colour intensity of the pigment in each treatment was measured using the Hunter colorimeter (CIELAB colorimetric system) at 500 nm. Stability tests were carried out following the method described by Suwannarach *et al*. (2019) with some modifications. Each treatment was performed in triplicates.

#### *2.5.1 pH stability*

One mg of purified pigment was diluted with 10 mL of 1N NaOH in a test tube. The solvent used was based on a previous study by Zhang *et al*. (2021) where the melanin pigment was found to be most soluble in 1 N NaOH. Then 0.1 N NaOH or 0.1 N diluted HCl was added to the pigment solution until pH 3.0, pH 7.0 and pH 15.0 were obtained. The solutions were incubated for 120 min at room temperature and the stability of the pigment solution was measured (Suwannarach *et al*., 2019). Measurements were taken at 0 min, 60 min and 120 min in triplicates.

#### *2.5.2 Heat stability*

Following the stability test of pigment under different pH concentrations, the pH with the highest pigment yield (pH 7.0) was chosen to determine heat stability. The pigment solution was incubated in a water bath at temperatures of 40℃, 60℃, 80℃ and 100℃ for 5 hours and the stability of the pigment solution was measured (Poorniammal and Gunasekaran, 2015; Suwannarach *et al*., 2019).

#### *2.5.3 Light stability*

The pigment solution was exposed under sunlight (from 11 am to 4 pm), and fluorescent light at a distance of 1 m with a light intensity of 2000 lx for 5 h to determine light stability (Suwannarach *et al*., 2019).

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*2.5.4 Antioxidant treatment*

One mg of purified pigment was diluted in 10 mL of 1 N NaOH and 0.02 mg of reducing agent (ascorbic acid, butylated hydroxyl anisole, sodium metabisulphite or citric acid) was added in a glass tube. The solution mixture was incubated at room temperature for 120 min and the stability of the pigment solution was measured (Poorniammal and Gunasekaran, 2015).

*2.6 Statistical analysis*

All the analyses and measurements were done in triplicates for each type of mushroom. The results were reported as mean value ± standard deviations and the obtained data were statistically analysed using one-way analysis of variance (ANOVA) by using the Statistical Package for Social Sciences (SPSS) software. Differences between means at 5% (P<0.05) level were considered significant.

## 3. Results and discussion

*3.1 Pigment stability between isolation and purification techniques*

Figure 2 shows the pigment powder isolated from *Termitomyces* sp., (termite mushroom) (Figure 2a), *Pleurotus citrinopileatus* (yellow oyster) (Figure 2b) and *Pleurotus djamor* (pink oyster) mushrooms (Figure 2c).

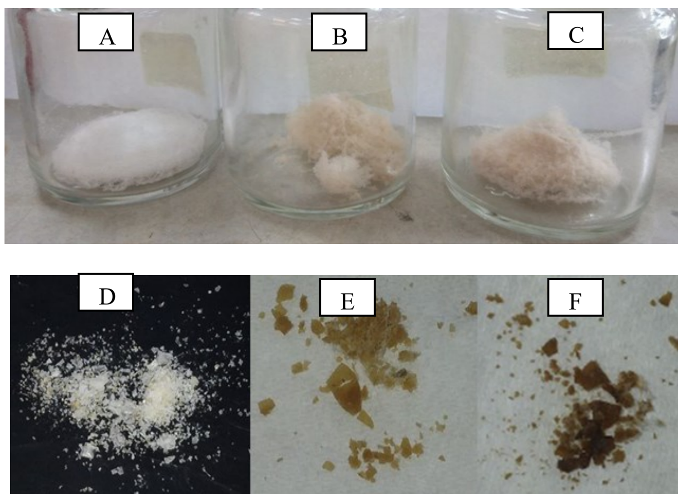


Figure 2. Images indicating pigment isolated from (a) termite mushroom (*Termitomyces* sp.), (b) yellow oyster (*P. citrinopileatus)* and (c) pink oyster (*P. djamor).* Subsequently, pigment purified from (d) termite mushroom (*Termitomyces* sp.), (e) yellow oyster (*P. citrinopileatus)* and (f) pink oyster (*P. djamor).*

Table 1 showed that there was significantly higher in lightness (L* values) of *Termitomyces* sp. pigment powder compared to *P. djamor* and *P. citrinopileatus*. On the other hand, there was no significant difference in redness (a* values) of all the isolated pigments. Yellowness (b* values) of the *P. djamor* and *P. citrinopileatus* pigments were significantly lower compared to *Termitomyces* sp. pigment. Utilisation of ethanol in the purification step allows suitable interaction between cell walls and pigment penetration which allows stability of pigment dispersion (Dan *et al*., 2009). Selection of solvents during purification step is crucial to determine the yield of pure pigment (Feghali and Nawaz, 2018). This study involved utilization of ethanol, chloroform, and ethyl acetate during purification step, that further allowed darker colour of the purified pigment compared to the isolated pigment. Purified pigments are presented in Figure 2d, Figure 2e and Figure 2f for *Termitomyces* sp., *Pleurotus citrinopileatus* and *Pleurotus djamor*, respectively. Apart from conventional pigment isolation and purification method using solvents, extraction system using ionic liquid has been used as an alternative method to obtain pigments (Lebeau *et al.*, 2020).

*3.2 Pigment stability towards pH and light*

Table 2a, 2b and 2c summarised the L* a* b* values of different mushroom pigment samples at different pH of 3.0, 7.0 and pH 15.0. There was a significant difference in the L* values for all pigment samples at pH 3.0, 7.0 and 15.0. The pigment solution of the samples turned darker as the pH increased. Alkaline pH helps to speed up the melanin polymerisation (Chen *et al.*, 2020). As indicated in Table 2a, pigment extracted from *Termitomyces* sp. (termite mushroom) showed significantly lower L* values at pH 15.0 compared to samples treated at pH 3, which represents darker shades of white pigment being obtained. The redness (a* values) of pink pigment extracted from *P. djamor* (pink oyster) was relatively stable at pH 3.0 and pH 7.0 (until 60 min) (Table 2b). However, the a* values were significantly lower at pH 15.0 compared to samples treated at pH 3 and pH 7, indicating that lighter shades of pink pigment was obtained. Meanwhile, Table 2c showed a darker shade of yellow pigment extracted from *P.*

Table 1. Difference between the colour values of isolated and purified mushroom pigment.

| | Isolated Mushroom Pigment | | | Purified Mushroom Pigment | | |
|---|---|---|---|---|---|---|
| Index/Colour values | L* | a* | b* | L* | a* | b* |
| *Termitomyces* sp. | 84.00±0.56[a] | 0.11±0.04[a] | 5.61±0.17[a] | 80.08±0.95[a] | 1.25±0.08[c] | 10.41±0.20[a] |
| *P. djamor* | 69.02±0.46[b] | 4.32±0.62[a] | 16.46±1.41[b] | 28.73±0.37[b] | 6.57±0.26[a] | 18.08±0.25[b] |
| *P. citrinopileatus* | 68.62±0.30[b] | 1.97±0.37[a] | 18.50±0.82[b] | 48.6 ±0.09[c] | 2.60±0.22[b] | 21.55±0.08[c] |

Data are presented as mean ± standard deviation. Means in the column with different superscripts are significantly different at p≤0.05. L*, color brightness (L* = 0 black, L*= 100 white); a*, green color (a*<0), red color (a*>0); b*, blue color (b*<0), yellow color (b*>0).

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Table 2a. Colour analysis of *Termitomyces* sp. pigment sample at pH 3.0, 7.0 and 15.0.

| pH | Index/ Colour values | *Termitomyces* sp. | | | % Discolouration |
|---|---|---|---|---|---|
| | | 0 min | 60 min | 120 min | |
| pH 3.0 | L* | 59.17±0.16[a] | 58.94±0.18[b] | 58.08±0.16[c] | 4.37 |
| | a* | 0.07±0.03[ab] | 0.05±0.02[a] | 0.10±0.02[b] | 30 |
| | b* | 2.32±0.10[a] | 2.43±0.08[b] | 2.47±0.05[b] | 6.08 |
| pH 7.0 | L* | 54.37±0.19[a] | 53.90±0.17[b] | 52.84±0.14[c] | 5.75 |
| | a* | 0.05±0.03[a] | 0.0±0.09[a] | 0.07±0.05[a] | 28.57 |
| | b* | 2.1 ±0.04[ab] | 2.07±0.01[a] | 2.09±0.01[b] | 0.96 |
| pH 15.0 | L* | 39.15±0.18[a] | 38.76±0.20[b] | 38.39±0.16[c] | 2.75 |
| | a* | -0.27±0.09[ab] | -0.24±0.04[a] | -0.20±0.02[b] | 35 |
| | b* | 0.74±0.03[a] | 0.72±0.06[b] | 0.78±0.4[b] | 5.13 |

Data are presented as mean ± standard deviation. Means in the column with different superscripts are significantly different at $p \leq 0.05$.
% Discolouration = (tan h after treatment)$^{-1}$ – (tan h before treatment)$^{-1}$ / (tan h before treatment)$^{-1}$

Table 2b. Colour analysis of *P. djamor* pigment sample at pH 3.0, 7.0 and 15.0.

| pH | Index/ Colour values | *P. djamor* | | | % Discolouration |
|---|---|---|---|---|---|
| | | 0 min | 60 min | 120 min | |
| pH 3.0 | L* | 48.03±0.12[a] | 47.81±0.10[b] | 47.63±0.09[c] | 1.41 |
| | a* | 1.58±0.06[a] | 1.53±0.01[a] | 1.41±0.04[b] | 12.06 |
| | b* | 3.96±0.11[a] | 3.91±0.08[a] | 3.87±0.12[a] | 2.33 |
| pH 7.0 | L* | 46.12±0.13[a] | 45.94±0.10[b] | 45.75±0.08[c] | 1.30 |
| | a* | 1.20±0.06[a] | 1.14±0.03[a] | 1.17±0.01[b] | 2.56 |
| | b* | 3.54±0.10[a] | 3.40±0.02[b] | 3.47±0.06[a] | 2.02 |
| pH 15.0 | L* | 34.08±0.11[a] | 33.75±0.16[b] | 33.96±0.10[c] | 0.45 |
| | a* | -0.18±0.03[a] | -0.15±0.06[a] | -0.17±0.02[a] | 5.88 |
| | b* | 1.52±0.10[a] | 1.42±0.07[b] | 1.48±.09[a] | 2.70 |

Data are presented as mean ± standard deviation. Means in the column with different superscripts are significantly different at $p \leq 0.05$.
% Discolouration = (tan h after treatment)$^{-1}$ – (tan h before treatment)$^{-1}$ / (tan h before treatment)$^{-1}$

Table 2c. Colour analysis of P. *citrinopileatus* pigment sample at pH 3.0, 7.0 and 15.0.

| pH | Index/ Colour values | *P. citrinopileatus* | | | % Discolouration |
|---|---|---|---|---|---|
| | | 0 min | 60 min | 120 min | |
| pH 3.0 | L* | 47.28±0.10[a] | 47.02±0.13[b] | 46.91±0.11[b] | 1.30 |
| | a* | 0.48±0.07[a] | 0.47±0.05[a] | 0.41±0.07[a] | 17.07 |
| | b* | 3.77±0.09[a] | 3.91±0.13[b] | 3.89±0.06[b] | 3.09 |
| pH 7.0 | L* | 45.01±0.11[a] | 44.80±0.08[b] | 44.72±0.12[b] | 1.01 |
| | a* | 0.41±0.09[a] | 0.34±0.04[a] | 0.38±0.06[a] | 7.89 |
| | b* | 3.28±0.08[a] | 3.32±0.04[a] | 3.37±0.02[b] | 2.68 |
| pH 15.0 | L* | 39.07±0.13[a] | 38.92±0.10[b] | 38.84±0.11[b] | 0.82 |
| | a* | -0.09±0.03[a] | -0.09±0.01[b] | -0.11±0.03[a] | 18.18 |
| | b* | 1.29±0.11[a] | 1.34±0.08[b] | 1.33±0.05[b] | 3.01 |

Data are presented as mean ± standard deviation. Means in the column with different superscripts are significantly different at $p \leq 0.05$.
% Discolouration = (tan h after treatment)$^{-1}$ – (tan h before treatment)$^{-1}$ / (tan h before treatment)$^{-1}$

*citrinopileatus* (yellow oyster) at pH 15.0. Wahyuningsih *et al.* (2017) reported that anthocyanins that contributes to redness of pigment is stable in acidic conditions. At different pH, pigments had different stability. This can result in colour changes to pink, red and yellow under acidic, neutral and alkaline conditions, respectively (Faraag *et al.*, 2017). The stability of pigment is important in food application. Understanding the pH of the natural pigments would enable its incorporation into various food products, such as dairy products (yoghurt and ice cream), candies, beverages and bakery products (Bocker and Silva, 2022). Recently, natural pigments have been applied in pH-sensitive active packaging that enables the detection of pH changes the food products during storage (Khan and Liu, 2022).

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Table 3. Colour analysis of pigment samples exposed to different source of light.

| | *Termitomyces* sp. | | | *P. djamor* | | | *P. citrinopileatus* | | |
|---|---|---|---|---|---|---|---|---|---|
| | L* | a* | b* | L* | a* | b* | L* | a* | b* |
| Control | 31.65±0.10$^{a}$ | -0.20±0.01$^{a}$ | 0.79±0.02$^{a}$ | 31.74±0.12$^{a}$ | -0.07±0.03$^{ab}$ | 1.43±0.04$^{a}$ | 31.22±0.06$^{a}$ | -0.05±0.01$^{a}$ | 1.14±0.03$^{a}$ |
| Sunlight | 31.66±0.07$^{a}$ | -0.18±0.01$^{a}$ | 0.75±0.02$^{a}$ | 31.86±0.24$^{a}$ | -0.11±0.07$^{a}$ | 1.45±0.05$^{a}$ | 30.89±0.58$^{a}$ | 0.20±0.26$^{a}$ | 1.13±0.03$^{a}$ |
| Fluorescent | 31.64±0.07$^{a}$ | -0.18±0.01$^{a}$ | 0.77±0.02$^{a}$ | 31.70±0.17$^{a}$ | 0.08±0.03$^{a}$ | 1.45±0.03$^{a}$ | 31.21±0.03$^{a}$ | -0.05±0.02$^{a}$ | 1.15±0.02$^{a}$ |

Data are presented as mean ± standard deviation. Means in the column with different superscripts are significantly different at $p \leq 0.05$. L*, color brightness (L* = 0 black, L*= 100 white); a*, green color (a*<0), red color (a*>0); b*, blue color (b*<0), yellow color (b*>0).

The pigment solution was subjected to different light sources (sunlight and fluorescence light) as shown in Table 3. It was observed that there was no significant difference in the colour intensity between the pigment subjected sunlight and fluorescent light exposure compared to control after 5 hours. This is supported by a previous study by Zou *et al.* (2015), which reported that *Auricularia auricula* fermentation broths melanin powder had little effect on under nature light.

3.3 *Pigment stability in varying temperatures and antioxidant levels*

As seen in Table 4 all pigment samples significantly changed from dark to lighter colour (for L* value and a* value) upon heating at 100°C. However, significantly darker yellow pigment (b* values) was detected after heating up till 100°C compared to samples at room temperature. The lightness (L* values) of white pigment samples from *Termitomyces* sp. showed significantly higher values after being heated at 60°C, 80°C and 100°C compared to samples at room temperature. The redness (a* values) of pink pigment extracted from *P. djamor* was significantly higher at 60°C compared to other temperature treatments. It was also observed that there was significantly higher in the yellowness (b* values) for the yellow pigment from *P. citrinopileatus* at room temperature compared to those heated at 40°C, 60°C, 80°C and 100°C. Changes in temperature influences structural changes of pigment, resulting in lighter shades of colour (Buresova *et al.*, 2023). The pigment colour contains chromophore in their structure that can absorb electromagnetic radiation that excite electrons and raise it to higher energy levels. These electrons usually return back to ground state and releases energy at a specific wavelength within the UV-Vis region, enabling colour to be visualised. Chromophores can be altered due to various reasons such as solvent polarity, targeted pigment, extraction time, temperature and agitation methods. The parameters involved allows disruption of structure and suitable penetration of cell walls allowing subsequent pigment isolation. At the same time, isolated pigment usually includes protein, lipids, antioxidants and sugar due to complex composition in mushroom. Hence stability of extracted pigment during temperature treatments and

Table 4. Colour analysis of pigment samples at different temperatures.

| Temperature | *Termitomyces* sp. | | | *P. djamor* | | | *P. citrinopileatus* | | |
|---|---|---|---|---|---|---|---|---|---|
| | L* | a* | b* | L* | a* | b* | L* | a* | b* |
| Room Temperature (Control) | 31.65±0.10$^{a}$ | -0.20±0.01$^{a}$ | 0.79±0.02$^{a}$ | 31.74±0.12$^{a}$ | -0.07±0.03$^{ab}$ | 1.43±0.04$^{a}$ | 31.22±0.06$^{a}$ | -0.05±0.01$^{a}$ | 1.14±0.03$^{a}$ |
| 40°C | 32.25±0.06$^{a}$ | -0.18±0.01$^{a}$ | 1.65±0.08$^{b}$ | 32.84±0.25$^{a}$ | -0.02±0.01$^{a}$ | 1.64±0.04$^{a}$ | 30.47±0.05$^{b}$ | -0.19±0.02$^{b}$ | 1.62±0.02$^{b}$ |
| 60°C | 28.04±0.16$^{b}$ | -0.08±0.02$^{b}$ | 1.36±0.08$^{b}$ | 31.11±0.12$^{a}$ | 0.18±0.02$^{b}$ | 1.47±0.04$^{a}$ | 29.21±0.18$^{c}$ | -0.19±0.01$^{b}$ | 1.31±0.03$^{c}$ |
| 80°C | 29.17±0.10$^{b}$ | 0.06±0.02$^{c}$ | 1.58±0.03$^{b}$ | 31.56±0.05$^{a}$ | -0.10±0.01$^{ab}$ | 1.90±0.06$^{a}$ | 30.66±0.05$^{ab}$ | -0.24±0.02$^{b}$ | 1.58±0.03$^{b}$ |
| 100°C | 52.94±1.65$^{c}$ | -0.77±0.06$^{d}$ | -1.83±0.32$^{c}$ | 61.12±1.61$^{b}$ | -0.50±0.12$^{c}$ | 7.38±1.30$^{b}$ | 59.20±0.48$^{d}$ | -0.67±0.09$^{c}$ | 1.72±0.05$^{d}$ |

Data are presented as mean ± standard deviation. Means in the column with different superscripts are significantly different at $p \leq 0.05$. L*, color brightness (L* = 0 black, L*= 100 white); a*, green color (a*<0), red color (a*>0); b*, blue color (b*<0), yellow color (b*>0).

Table 5. Colour analysis of pigment samples treated with antioxidants usually used in food industry.

| | *Termitomyces* sp. | | | *P. djamor* | | | *P. citrinopileatus* | | |
|---|---|---|---|---|---|---|---|---|---|
| | L* | a* | b* | L* | a* | b* | L* | a* | b* |
| Control | 30.80±0.47$^{a}$ | 0.19±0.04$^{a}$ | 1.04±0.27$^{a}$ | 31.29±0.65$^{a}$ | -0.06±0.02$^{ab}$ | 1.27±0.11$^{a}$ | 31.20±0.28$^{a}$ | -0.03±0.15$^{a}$ | 1.02±0.16$^{a}$ |
| Ascorbic acid | 29.67±0.53$^{b}$ | 0.12±0.04$^{a}$ | 0.71±0.13$^{a}$ | 31.46±0.20$^{a}$ | 0.08±0.05$^{a}$ | 0.55±0.06$^{b}$ | 32.73±0.16$^{b}$ | -0.25±0.08$^{ab}$ | -0.06±0.04$^{b}$ |
| Citric acid | 28.41±0.31$^{c}$ | -0.20±0.03$^{b}$ | 0.78±0.15$^{a}$ | 29.45±0.12$^{b}$ | -0.17±0.05$^{b}$ | 1.46±0.04$^{c}$ | 25.63±0.44$^{c}$ | -0.32±0.06$^{b}$ | 2.13±0.17$^{c}$ |
| BHA | 28.86±0.21$^{bc}$ | 1.14±0.08$^{c}$ | 0.70±0.06$^{a}$ | 26.88±0.12$^{c}$ | -0.94±0.13$^{c}$ | 1.43±0.05$^{ac}$ | 31.64±0.25$^{a}$ | 0.41±0.17$^{c}$ | 1.01±0.13$^{a}$ |
| Sodium metabisulphite | 28.35±0.35$^{c}$ | -0.34±0.19$^{b}$ | 0.69±0.11$^{a}$ | 31.57±0.23$^{a}$ | 0.07±0.04$^{a}$ | 0.61±0.03$^{b}$ | 26.77±0.23$^{d}$ | -0.18±0.06$^{ab}$ | 1.81±0.12$^{c}$ |

Data are presented as mean ± standard deviation. Means in the column with different superscripts are significantly different at $p \leq 0.05$.

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antioxidants needs to be explored since mushroom may reduce extracted pigment yield or colour intensity (Bocker and Silva, 2022).

As seen in Table 5, the yellowness (b* values) of white pigment solution from *Termitomyces* sp. showed no significant differences between the control, and those treated with antioxidants commonly used in food industries. However, the lightness (L* values) of the white pigment solution showed significant differences in the intensity of colour between the control and samples treated with antioxidants. Pink pigment redness (a* values) was stable and showed no significant differences with ascorbic acid, citric acid and sodium metabisulphite treatments compared to control. Interestingly, antioxidants increased darker shades of white pigments (L* values) from *Termitomyces* sp. compared to control samples. It was postulated that antioxidants available in mushrooms may affect pigment colour intensity and purified pigment yield (Bocker and Silva, 2022). However, the pink pigment solution extracted from *P. djamor* showed greater stability to antioxidants and showed no significant differences in colour intensity for redness (a* values) except for BHA. On the other hand, the yellow pigment (b* values) from *P. citrinopileatus* showed greater stability when treated with BHA compared to other antioxidants.

## 4. Conclusion

In conclusion, the isolation of pigment from *Termitomyces* sp., *P. djamor* and *P. citrinopileatus* produced a white, pink and yellow colour pigment powder respectively. The purification process intensified the pigment colour. All purified mushroom pigments showed greatest stability at pH 7.0. The *Termitomyces* sp. were stable at 40°C, however, *P. djamor* pigments were stable at 40°C, 60°C and 80°C. Meanwhile *P. citrinopileatus* pigment was unstable to heat treatments. The colour intensity of any of the pigment samples from mushrooms did not alter when exposed to light. Yellow pigment was more stable against BHA than other antioxidants while the mushroom pigments (white and yellow) shown stability against various food antioxidants (ascorbic acid, citric acid and sodium metabisulphite). By using mushroom pigments and considering processing parameters like temperature, pH, additional antioxidants, and light sources that may eventually interfere with the qualities of any final food product, the food industry will be able to build new goods.

## Conflict of interest

The authors declare no conflict of interest.

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