Effects of Brazilin Contained in *Caesalpinia sappan* L on The Properties of Fish Paste from Ponyfish (*Leiognathus* sp)

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**Abstract:** Fish paste is one of fisheries products made by fermentation of small shrimp or non economically fishes and became popular in Indonesia as a condiment related rich in umami taste. Fish paste from different region have not same appearance and nutritional as result of processing variety such as raw material, composition and fermentation time. Most people think that the reddish brown fish paste color tends to be more attractive than the brown or blackish brown ones. This study aims to investigate the effect of brazilin extract of the dried heartwood of *Caesalpinia sappan* L (BEC) on the physiochemical properties of fish paste from ponyfish (FPP). The powder of BEC was extracted using solution contain 96% ethanol and 1% HCl with ratio of the dried heartwood (in sawdust form) and solution are about 1:5. FPP was produced using three different concentration of BEC: 5%; 7.5%; 10% and without BEC (0%) as a control treatment. Data were analyzed using Analysis of Variance and Kruskall-Wallis. Addition of the 5% and 10% BEC in fish paste resulted in lower lightness and higher redness compared with control (p<0.05). The 7.5% of BEC resulted significant difference (p<0.05) to color compared other treatments with Hue value test ranges from 18-54° (indicates high red color). The other parameters such as moisture content, amount of Lactic Acid Bacteria and sensory attributes did not show any difference. In conclusion, the 7.5% of BEC exhibited the effectiveness in color performance and sensory attributes in FPP.

1. **Introduction**

Fish paste is a flavor enhancer in a paste form, specific fermented aroma based on fishes, shrimps used or mixture of both with addition of salt and other additives. Fish paste is a popular taste enhancer in many dishes in Indonesia [1], well known as *terasi*, a popular condiment made from fermentation of fish, shrimp or mixed of both, which has a sharp and unique aroma as a normally fermented fish product [2]. Fish paste has reddish brown to blackish brown color depending on the raw materials and the spontaneous occurring microorganism which involve during fermentation [2]. The other study stated there are two kinds of commercial fish paste, shrimp paste which has brownish color and finfish fish paste that has light brown and darkish color [3]. Most people think that the reddish-brown fish paste color tends to be more attractive than the brown or blackish brown ones. This thought leads some of fish paste’s processor, which most of them are coastal communities who are unfamiliar with knowledge of food safety, made fish paste in an incorrect way by using artificial colorant used for textile, known as Rhodamine B, Rhodamine B (RhB) and Rhodamine 6G (R6G), derivatives of xanthene pigments, have been play roled in printing industries due to their deeply pink color [4, 5]. However, these are commonly poisonous and dangerous to humans and animals. According to a research by Amir and Mahdi [6], 60% of commercial fish paste contains 11.81-19.05 ppm of Rhodamine B. This condition was very alarming considering that fish paste is a very popular condiment. If the harmful dyes in fish paste and consumed...
by the communities, it can cause the problem of asthma, chromosome damage and other disease, so it has been strictly banned as a food ingredient in many countries [7].

Pony fish is a non-economically fish which are landed as by catch in large amount [8]. Ponyfish has a good chemical composition, the protein content is the second most abundant after its moisture content. According to Öziyurt et al. [9], protein content of raw klunzinger’s ponyfish is up to 79.97% (in freezedried sample), indicated that these are suitable for supplementation as an ingredient for human consumption. Protein as an ingredient resulted from low-cost source is relevant for the industry. Moreover, protein hydrolysate of ponyfish can serve as possible ingredient for a healthy need for human protein that are easier to absorb into the body than intact proteins [10]. Unfortunately, fish paste resulted from ponyfish as a raw material has not an intense color. Therefore, naturally derived compound needed to solve the color problem in fish paste.

A lot of researchers have analyzed the naturally occurring colorant agents of Caesalpinia sappan L. extract for enhancing attractive color in fish paste products. C.sappan L., found in Southeast Asia, it has been used as oriental medicines due to its biological activities and phytochemical components, such as flavonoids, lignans, steroids, triterpenoids, and diterpenoids. Recently, it has also been stated that the oxidative stability of meat products has been effectively enhanced [11]. Specifically, no research has yet investigated the appearance in fish paste products that occur when using C. sappan L. extract as a natural ingredient or as a colorant substitute. The utilities of heartwood C.sappan L extract was able to give a better color performance on ponyfish paste (fish paste). It is due to heartwood contains a colorant substance known as brazilin which has a purplish red color. Homoisoflavonoid compounds, such as brazilin, are the main phytochemical components of sappan wood. In addition, sappan wood also produces derivatives of dibenzoxin such as protosappanin and its isomers [12]. The most widely used component of sappan is heartwood, which includes water-soluble dyes such as brazilin, protosappanin, sappan chalcone and hematoxylin [13]. The purpose of this study was to investigate the effects of brazilin found in the C. sappan L. extract (BEC) on properties of fish paste from ponyfish (Leiognathus sp) (FPP).

2. Methods

2.1. Material

Material used for this study were consisted of two parts. First extraction of brazilin from C. sappan L. (BEC), as follows: the heartwood of C. sappan L is obtained from traditional market in West Kalimantan; ethanol 96%; HCl 37% and gelatin. Secondly, for the processing of fish paste from ponyfish (FPP), fresh ponyfish, salt and banana leaf are purchased from traditional market in West Kalimantan.

2.2. Preparation of Brazilin Extracts from C. sappan L. (BEC)

BEC was extracted as described by Holinesti [14] with some modification. BEC was prepared by grinding the heartwood of C. sappan to obtain sawdust form. Then, the sawdust was weighing and mixing with solvent contains ethanol 96% acidified with 1% of HCl 37% five volumes from the amount of the sawdust (1:5 w/v). The extract was then filter using filter paper. The following treatment was adding 0.5% gelatine and let it settle for 12 hours. Then the solution was filtered and centrifuged at 4000 rpm for 15 minutes.

2.3. Production of Fish Paste

In the processing of fish paste, referred to Karim et al., [15] with some modification. Fresh ponyfish are selected, cleaned, drained and dried until half dry. Upon drying, filths such as small fish, mussel shells and coral are removed. After that, semi dried fish is weight, added with salt (10% from half dried ponyfish), and grinded to tenderize fish meat for making into paste or dough. After stored overnight, the dough was sun-dried for 2-3 hours. The sun-dried dough was then grinded for a second time and added with BEC as treatment as follows: 5%; 7.5% and 10%, and also fish paste without BEC (0%) as a control treatment. After that, the grinded dough was sun-dried for 3 hours and then packaged with banana leaf and kept at ambient temperature for 30 days.

2.4. Physiochemical Analysis
Moisture content was analyzed according to Association Official of Analytical Chemists [16]. Hedonic test referred to Meilgaard et al. [17] and the International Organization for Standardization (ISO) [18], twenty-five (25) semi-trained panelists are students of Fish Products Technology Department, Diponegoro University, the age of panelist between 19 - 21 years old. These panelists had received 25 g of each terasi sample. The value for the overall taste/flavor acceptability/liking of each fish paste was calculated by using a hedonic scale, i.e. 1 (dislike quite much); 2 (dislike mildly); 3 (dislike slightly); 4 (neither displeasure nor like); 5 (like slightly); 6 (like moderately); 7 (like quite much). Panelists were asked to select a sample they liked the most, based on appearance (fish paste color) and taste. The taste of fish paste was characterized by panelists as fishy. The word “foreign flavor” was used by panelists to describe the presence of flavors that were not usually associated with fish paste.

2.5. Color Measurements [19]
The colors L* (lightness), a* (redness) and b* (yellowness) of fish paste samples were tested using the Minolta CR 300 colorimeter (Minolta, Tokyo, Japan). The system was calibrated with a white standard plate (Y=93.5; x=0.3132; y=0.3198) for triplicate calculation of each fish paste sample. The value of chroma (C*) and hue angle (h°) were determined using Eqs. (1) and (2) as sugested by Minolta (1994).

\[ C^* = \sqrt{a^*2 + b^*2} \]  
\[ h° = \tan^{-1}(b*/a*) \]

2.6. Microbial Analysis [20]
Microbial analysis of fish paste was carried out immediately after finished the fish paste production, sample were analyzed for lactic acid bacteria (LAB) count. Five grams of minced samples were aseptically combined with 45 mL of sterilized saline with 0.85% sodium chloride in a sterile plastic bag. The mixture was homogenized in a stomacher (BM-400P, Fish Product Technology Laboratory, Undip, Indonesia) for 2 min. Suitable decimal dilutions were prepared in sterile saline (0.85%), and 1 mL of the required diluted solutions was poured into the de Mann Rogosa and Sharpe (MRS) agar (CM 0361, OXOID) plates for LAB and incubated at 30 °C for 48 h. MRS agar contains polysorbate, acetic, magnesium, and manganese which are known to react or act as growth factor of Lactobacillus. Fish paste sample was diluted and isolation was done using pour plate technique. Isolation was then done to obtain pure LAB isolates. The findings were presented with logarithms of the number of colony-forming units per gram (log CFU/g).

2.7. Statistical Analysis
The experimental design was a fully randomized design of three replicates. For statistical analysis results means with a standard mean error were generated from three replications. The Excell software was implemented for data analysis and the Variance Analysis (ANOVA) technique was conducted in this study. Differences between means at 95% of the significant level were measured using an Honestly Significant Different test.

3. Results and Discussions

Moisture content of Fish Paste
Moisture content on fish paste with addition of Brazilin Extract from Caesalpinia sappan L. (BEC) is shown on Table 1.

| Treatments       | Moisture content (%) |
|------------------|----------------------|
| Without BEC (Control) | 35.46 ± 0.15a       |
| 5% BEC           | 35.21 ± 0.16a       |
| 7.5% BEC         | 34.33 ± 0.16a       |
| 10% BEC          | 35.54 ± 0.18a       |

Means with different letters are statistically significant (p < 0.05)
The moisture content of the fish paste was 34.33-35.54%. On the basis of the performance, it can be shown that there are no major differences with addition of BEC on fish paste. This is probably because the same composition of fish and salt were used in the making of fish paste and differ only in the amount of BEC. Additionally, BEC added into fish paste was in form of dried powder. The moisture content of fish paste in present study in line with the study conducted by Daroonpunt et.al [21] which stated that kapi (shrimp paste) from Thailand has a moisture content of between 33.95-52.19%. The moisture content of fermented fish products differed due to variations in the fermentation period and drying phase of the product. The outcome of this analysis was also a higher moisture content compared to acan (traditional shrimp paste from Maduranese, Indonesia) [22]. The moisture content of the fish paste indicates that fish paste can be graded as an intermediate moisture food, that it can be combined with an increase in shelf life and protect the product from spoilage microorganism at ambient temperature [23; 24].

**Hedonic of Panelist on Fish Paste**

The results of panelist hedonic levels on fish paste is shown in Table 2.

| Treatments | Hedonic score * | Σ Like the most | Σ Dislike the most |
|------------|----------------|----------------|-------------------|
| Without BEC (Control) | 3.78 ± 1.34a | 2 | 11 |
| 5% BEC | 3.64 ± 1.56a | 6 | 7 |
| 7.5% BEC | 4.26 ± 1.68b | 10 | 3 |
| 10% BEC | 4.43 ± 1.36b | 7 | 4 |

N=25 panelists; hedonic score: 1 (dislike quite much); 2 (dislike mildly); 3 (dislike slightly); 4 (neither displeasure nor like); 5 (like slightly); 6 (like moderately); 7 (like quite much). a All data are presented as the mean ± SD of 25 panelists. Means with different letters are statistically significant (p < 0.05).

The acceptability of fish paste varies significantly (p < 0.05) within 4 samples. Panelist liked fish paste with 7.5% and 10% of BEC (scored > 4); whereas fish paste without BEC and with 5% of BEC were unacceptable (scored < 4). It is assumed that 7.5% of BEC contained in fish paste caused the fish paste colors more attractive compared with other treatments. Moreover, the same amount of fish that used in fish paste affected the aroma which probably decreased with increasing of BEC’s concentration especially in 10% of BEC. The personal experiences, familiarization could have affected the consumer judgement, preference and choice towards a certain food [25, 26, 27].

**Color of Fish Paste**

The various treatments for fish paste items had different colors, L*(lightness), a*(redness) and b*(yellowness) ranged from 38.56-45.68, 3.15-5.85 and 1.04-3.01, respectively (Table 3).

| Treatments | Lightness (L*) | Redness (a*) | Yellowness (b*) | Hue angle (h°) | Color of Fish Paste |
|------------|----------------|--------------|----------------|----------------|---------------------|
| Without BEC (Control) | 45.68±0.71a | 3.15±0.05a | 1.04±0.06a | 18.62±0.26a | Yellow red |
| 5% BEC | 41.11±0.24b | 4.54±0.18b | 2.08±0.01b | 25.71±0.10b | Reddish brown |
| 7.5% BEC | 41.05±0.23b | 4.94±0.09b | 2.05±0.00b | 28.50±0.01c | Reddish purple |
| 10% BEC | 38.56±0.21c | 5.85±0.00c | 3.01±0.00c | 28.98±0.00c | Reddish purple |

Means with different superscript within the same column indicate statistically significant (p < 0.05).
These samples were brown to red/purple in color. The variation in color may be caused by the difference between BEC and the added coloring agents, since the main raw material (fish and salt) are at the same concentration. Grinded, sun-dried and also fermentation stage in the development of fish paste leads the fish paste to a dark brown color. Aldehyde and ketone carbonyl groups, oxidation products, can react with amino groups of free amino acids or peptides formed during hydrolysis, leading to yellow or brown color progress [28]. In addition, fish paste prepared with 10% of BEC, showed higher a* values (5.85±0.00) compared to control (p < 0.05). This possibly happened because the highest concentration of brazilin extract in fish paste, which is gave reddish brown color of fish paste. In this study, L* value tends to decreased with increasing concentration of BEC. This phenomena, similar with studies were observed by Jin et al. [29] and Yim et al. [30] have acknowledged the application of C. sappan L. extract in sausages contributed to a decrease in the L* values at the time of storage. Control and fish paste with 5% of BEC showed a substantial improvement (p < 0.05) in yellowness (b*) and hue angle (h°) compared to 7.5% and 10% of BEC. Due to the variable values of CIE L*, h°, the unfaded color was retained and also considered to be appropriate to the panelist for fish paste, it is believed that 7.5% of BEC may be used as an alternative of natural coloring agents for fish paste.

**Lactic Acid Bacteria (LAB) Count of Fish Paste**

Lactic acid bacteria (LAB) count of fish pastes varied from 6.00 to 6.75 log cfu g⁻¹ in all samples (Table 4) and has no significant difference between treatments (p < 0.05).

| Treatments | LAB (log cfu g⁻¹) |
|------------|------------------|
| Without BEC (Control) | 6.75 ± 0.03a |
| 5% BEC | 6.75 ± 0.02a |
| 7.5% BEC | 6.76 ± 0.03a |
| 10% BEC | 6.00 ± 0.02a |

Means with different letters are statistically significant (p < 0.05).

The addition of BEC in fish paste had no effect to the bacteria counts. The counts of LAB reached a peak levels of 6.76 log cfu g⁻¹ for fish paste with 7.5% of BEC and decreased with 10% of BEC. The decreased of LAB at fish paste with 10% of BEC is probably associated with 10% of brazilin extract which reduced macro component such as protein, lipid and carbohydrates. In fermentation process, degradation of protein, lipid and and carbohydrates-produces certain flavoring compounds or their precursors and to some degree influences the consistency and taste the products. In this scenario, the carbohydrates used as growth media for LAB are likely to decreased with a rise in the BEC’s concentration in fish paste. These findings are consistent with the previously published results of the LAB count on fish-chilli paste are range from 6 to 8 log cfu g⁻¹ after 1 week fermentation and indicates there are reduction of fermentable carbohydrates [20]. LAB contained in fish paste indicates that this is a good products from the viewpoint of high nutrient sources and suitaed with study reported by Jockers [31], as described in fermented product, *Lactobacilli* had a complex nutrient and requirement as indicator of several assays, including the amount of amino acids in food products.

4. **Conclusions**

The present study concluded that brazilin extract from *Caesalpinia sappan* L. potentially applicable to enhance the color of fish paste. In detail, 7.5% of brazilin extract showed the most attractive appearance and flavor from the viewpoint of sensory attributes. In summary, the positive effects of brazilin extract addition from *C. sappan* L. in color properties of fish paste have been observed. Therefore, we concluded the brazilin extract can be built on an industrial scale to the future of conventional scale industries such as fish paste.
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