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## The Potency of betacyanins extract from a peel of dragon fruits as a source of colourimetric indicator to develop intelligent packaging for fish freshness monitoring

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**Abstract.** A Famous freshwater fish in Indonesia is *Osphronemus gouramy* (O. gouramy). This fish has a thick meat and savory taste. Recently, many researches have focused on developing the intelligent packaging for detecting spoilage fish. The aims for this research is to develop smart packaging through immobilizing betacyanin into glucomannan-Polyvinyl alcohol matrix used to monitor fish freshness. The color stability of matrix films was monitored using camera. Furthermore, the colourimetric films were used to observe freshness based on a TVB-N level of gourami (O. gouramy) on chilling temperature. The incubation result of betacyanin extracts with variation pH shows that the color change of betacyanin extracts at pH 10 and pH 12 are purple and yellow. Stability of colorimetric films color is reported that the colorimetric films are more stable after incubation on chilling temperature until 7<sup>th</sup> day. Evaluation of fish freshness of *Osphronemus gouramy* (O. gouramy) fillet was conducted at chilling temperature. Transformation of a colorimetric film becomes yellow after gourami fillet is, where it's simultaneously with increase of the level of TVBN for *O.gouramy* fillet is 39,74 mg/100g. Thus, it can be concluded this color change corresponded with TVB-N level are increased.

### 1. Introduction

Fish is one of the natural food which has a short-term of shelf life because it has heavy protein and water content. Good handling practice is needed to reduce the fish spoilage during supply chain. TVB-N level is one of the parameters to measure Fish freshness. Generally, the analysis of TVB-N level needs the technician's advanced skill to handle the instrumentation such as kjehdal intrumentations [1].

Intelligent packaging is a novel packaging which has ability to monitor food quality with the real-time conditions. Intelligent packaging such as one package containing matrix films cross-linked with a colorimetric indicator to monitor pH, temperature or freshness of some products. Fish spoilage was monitored with smart packaging used chemosynthetic as colour agents are reported by Kuswandi, et al [2] and Paquet et al [3]. Recently, a source of a colorimetric agent from a natural pigment to develop a smart packaging was investigated such as anthocyanin. Some advantages of the anthocyanin use are most obtainable in flesh, peel, leaf, or of fruits, and water soluble. Choi et al [4] and Ma & wang [5] suggested that colorimetric films used anthocyanin were important source to evaluate fish freshness

based-on ammonia gases production during fish spoilage process. It is widely recognized that anthocyanin is a safe ingredient to blend with food or beverage.

Currently, dragon fruits are <sup>19</sup>st commonly found in Indonesia, Malaysia, Taiwan, Australia, Colombia, <sup>14</sup>nam, and Mexico. It has been reported that the antioxidant activity of dragon fruit was  $22.4 \pm 0.29$  measured by DPPH method at E<sup>10</sup>, the vitamin C equivalent was  $118 \pm 4.12$   $\mu\text{mol}$  per g of peel dried and flesh extract, and flesh has total phenolic contents was  $42.4 \pm 0.04$  mg of gallic acid equivalents (GAE)/100 g of fresh weight. Moreover, betacyanins (betanin equivalents) content of flesh and peel were  $10.3 \pm 0.22$  and  $13.8 \pm 0.85$  mg per 100 g of fresh weight, respectively [6].

The aims for this research is to develop smart packaging through immobilizing betacyanin into glucomannan-Polyvinyl alcohol matrix. The micrograph of colorimetric films was recorded using scanning electron microscope. The color stability of matrix films was monitored. Furthermore, the colourimetric films were used to observe freshness based on a TVB-N level of gourami (O. gouramy) on chilling temperature.

## 2. MATERIAL AND METHODS <sup>21</sup>

### 2.1. Materials

Dragon fruits were harvested from farms of Jember Residence, Indonesia. A fully mature and no physical damage of dragon fruits were selected for study. Gouramys were obtained from local pool of Jember Residence, Indonesia. The chemicals used in this study were NaOH (Merck, Indonesia), HCl (Merck, Indonesia), glucomannan (CV. Nura jaya, Indonesia), polyvinyl alcohol and (sigma-aldrich, Singapore).

### 2.2. Methods

#### 2.2.1 Extraction of betacyanins

Solution was prepared by extracted a 50 gr of fresh red pitaya peel with 50 mL boiled-distilled water until 5 minutes, after that this solution was filtered.

#### 2.2.2 Quantification of Betacyanin Content

After preparing extraction solutions, the betacyanin content was <sup>6</sup>calculated according to with : <sup>13</sup> $[\text{BC} (\text{mg}/100 \text{ g}) = (\text{A} \times \text{DF} \times \text{MW} \times \text{V} \times 100) / (\epsilon \times \text{L} \times \text{W})]$ , where MW is molecular weights of betanin (550 g/mol), A is absorbance at 538 <sup>14</sup>nm and corrected at 600 nm, DF is the dilution factor, <sup>16</sup>L is the path length of the cuvette, V is sample volume (mL), W is the weight of dry matter in gram, and  $\epsilon$  is the molar extinction coefficients of betanin ( $60,000 \text{ L mol}^{-1} \text{ cm}^{-1}$ ) [7].

#### 2.2.3 Preparation of fish freshness fi<sup>23</sup>

1 g of PVA and 2 g of glucomannan mixed with 70 mL distilled water, and the mixture was boiled on 100 °C and blended used a magnetic stirrer until a formed homogeneous mixture. after that adjust pH for mixture until pH 8.5, 30 ml of betacyanin extract solutions was then added. Furthermore, the film was created by casting solution into a pan with a dimension of 5 x 10 cm. A pan was dried at 40°C for 36h in oven dryin (modified Zhai et al. [1]).

#### 2.2.4 Color stability of colorimetric films evaluation

Color stability of colorimetric film was evaluated visually during 7<sup>th</sup> days based on L, a, b values using MiniScan EZ 4500L, Hunter lab, virginia, USA (Hunt et al, [8]).

#### 2.2.5 Betacyanin Extracts spectral

<sup>16</sup>acyanin solution incubated at different pH (2, 4, 6, 8, 10, and 12), after that evaluate the spectra using a UV-Vis Spectrophotometer (Ultra Spec 2100 pro, amersham biosciences, US).

#### 2.2.6 Surface Profile of Colorimetric Films

Micrograph of the colorimetric films was recorded by SEM (TM-3030, Japan)

### 2.2.7 Trial of Fish Spoilage

100 g of fish fillet was immediately displaced into a plastic box. The matix films were installed into headspace of the plastic box (25 x 25 cm). The plastic box was incubated on a chilling temperature (4°C) until 8<sup>th</sup> days. Using camera to evaluate the color change of the colorimetric films every 24 h [1].

### 2.2.8 Quantification of Total Volatile Basic Nitrogen (TVB-N)

Determination of TVB-N value has used automatic distillation methods [9]. Where, 20 g of fish meal mixed with 40 ml of 7.5% TCA solutions, 5 ml filtrate was collected into the distillation tube. automatic distillation used to produce steam (Vapodest50s, Germany), and gasses were exposed with boric acid (0.1 N). TVB-N was calculated following by :

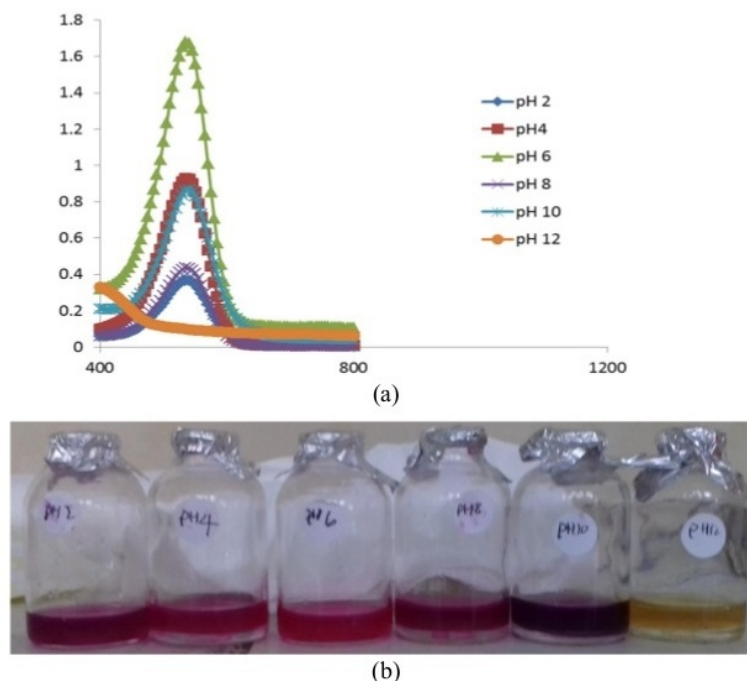
$$\text{TVB (mg/100 g)} = \frac{(V_{\text{sample}} - V_{\text{blanko}}) \times N_{\text{HCl}} \times 14,007 \times 100}{\text{Weight sample} \times \text{fp}} \quad (1)$$

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## 3. Results and Discussion

### 3.1 Spectral and Color of Betacyanins

UV-vis spectra of betacyanin solutions compared with the color changes is illustrated in Figure. 1. That result shows that betacyanin solutions color was purple on pH 2 until pH 10, the color change of betacyanin extract to yellow after incubated at pH of 12.



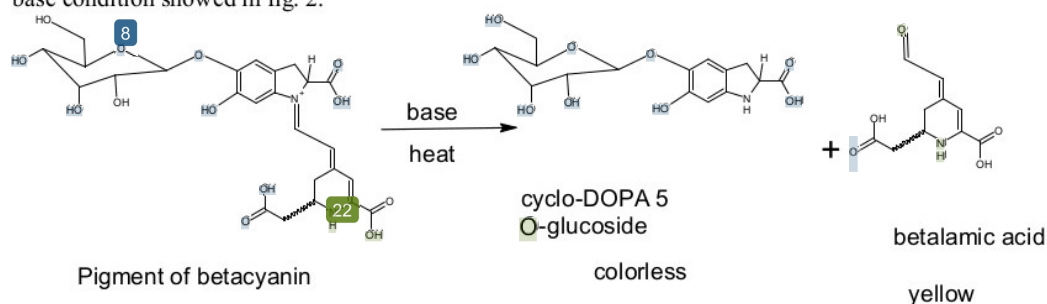
**Figure 1.** (a) UV-vis Color and (b) Spectral of betacyanins solution at pH 2, 4, 6, 8, 10 and 12.

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On the pH 6 it shows that the maximum absorbances was 538 nm. This was in according to the study of Fernanda Robert de Mello et al. [10] who reported that betalain extract solution from red



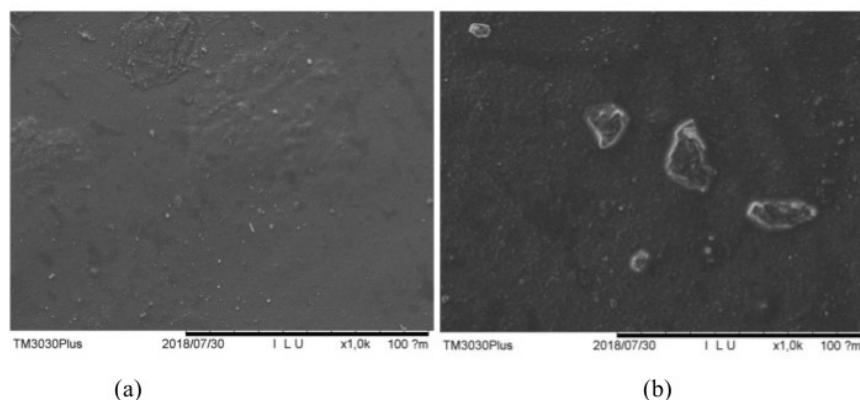
pitaya peel had maximum absorption peak at 540 nm. Additionally, it was found that the maximum absorption peak altered to 400 nm (yellow color) after the pH value of 12. Previously, Herbach et al. [11] reported that the betalains turned into yellow products such as betalamic acid (424 nm) after exposed to high temperature. The mechanism reaction of color change of betacyanin after incubated at base condition showed in fig. 2.



**Figure 2.** Mechanism reactions of the color change of betacyanin [12].

### 3.2 SEM Micrographs Analysis

Before preparation of colorimetric films, the level of betacyanin extracts for indicator solution was 119±0.71 mg/100 g. Then, this was used for developing glucomannan-PVA-betacyanin. The micromorphology of a glucomannan-PVA-betacyanin film and Glucomannan-PVA films is showed in Figure 3. This result showed that the glucomannan-PVA film forms a two-phase structure due to separation of glucomannan and PVA. Similarly, Cano et al, [13], and Zhai et al, [1] reported that a two-phase structure formed due to the degree of immiscibility of PVA. In the contrast, in presence of glacial acetic acid, starch/PVA shows a homogeneous mixtures or a single-phase. Starch/PVA films became elastic after incorporating a plasticizer such as CaCl<sub>2</sub> [14]. After that, the micrograph result showed that betacyanins extracts were successfully trapped into polymer matrix.



**Figure 3.** Micromorphology of (a) glucomannan-PVA-betacyanin film, (b) glucomannan-PVA film).

### 3.3 Stability of the colorimetric films color

Mostly, natural pigment has short shelf life is extract of betacyanin. This depends on the various factors such as pH, medium extractions and temperature. It has been reported that betacyanin extract in water corresponding to the first-order kinetic model with its self-life ( $t_{1/2}$ ) was 90% shelf life of 76

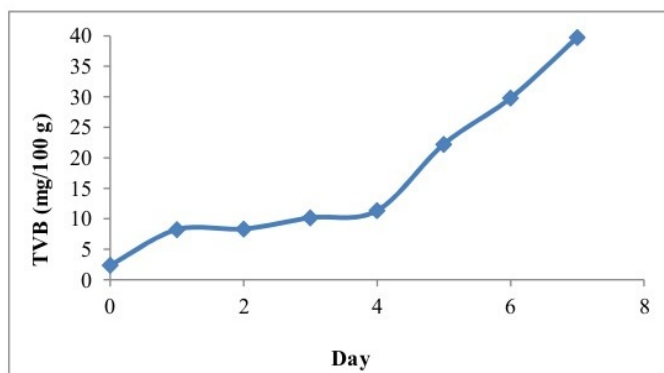
hours and 23 hours at 25 °C [15]. However, starch-PVA matrix probably inhibited oxidation process of the betacyanins [16]. The color stability of betacyanin-glucomannan-PVA is presented in Table 1. This result suggested that there was no significant color change on film during incubation at 4°C. On the other hand, Zhai et al.[1] reported that anthocyanin-glucomannan-pva matrix stored into chilling temperature at 4 °C had great color stability.

**Table 1.** Value of L, a, b, after incubation until 7<sup>th</sup> days at 4°C.

Day	L	a	b
0	40.935 ±0.22	67.12 ±0.41	-33.22 ± 0.056
1	41.02±2.72	43.98 ±2.80	-14.815 ± 0.01
2	41.895 ±1.12	44.875 ± 0.78	-15.16 ± 0.06
3	42.045 ±0.42	43.98 ± 0.077	-14.315 ±0.01
4	39.725 ±0.06	44.265 ±1.08	-13.175 ± 0.04
5	39.225 ±0.14	41.885 ± 1.08	-13.945 ±0.38
6	39.305 ± 0.14	40.255 ±0.06	-12.225 ±0.134
7	39.38 ±0.05	39.93 ± 0.084	-12.225 ±0.134

### 3.4 Evaluation Fish Freshness Using Colorimetric Film

The transformation of indicator films color are shown in Figure 3. In the beginning, the color of glucomannan-PVA-betacyanin film is purple. Furthermore, the color became yellow after 8 days. This color changed due to the increasing TVB-N level from gouramy. The color change due to interaction or the film exposed with gases such as NH<sub>3</sub>, H<sub>2</sub>S, where is before explained. Initially, TVBN value of gouramy was 2.4 mg/100 g. After eight days, TVB-N exceeded the standard values (39.74 mg/100 g). On the contrast, it has been reported that anthocyanins from roselle [1], purple sweet potato [4], and red cabbage [17], Zhang, Lu, and Chen [18] correspondingly with pH changes or the level of freshness matter. Thus, the results are suggested that the glucomannan-PVA-betacyanin can be used to evaluate fish freshness.



(a)



(b)

**Figure 4.** (a) TVB-N value of gouramy after stored on 4 °C until 8 days and (b) transformation of the colorimetric films.

**Table 2.** L, a, b, value of colorimetric films after applied on monitoring fish freshness until 8<sup>th</sup> days at 4°C.

Day	L	a	b
0	40.935±0.22	67.18± 0.48	-33.245±0.09
1	39.16± 0.08	60.615±0.7	-27.565±0.64
2	39.075±0.035	54.875 ±0.78	-15.095±0.106
3	34.825±3.17	43.98±2.8	-9.535±0.57
4	38.625±1.49	44.195±0.23	-13.205±0.06
5	46.955±0.035	29.85±0.46	-0.065±0.68
6	46.405±0.926	12.02±0.056	12.225±0.13
7	44.185± 0.035	24.02±2.77	13.34±0.084
8	45.295± 2.97	6.075±0.46	24.52±1.35

#### 4. Conclusion

The Result showed that the color stability of colorimetric films was considerably stable until 7 days at 4 °C. This result exhibited that the glucomannan-PVA film showed not homogen due to the separation of glucomannan and PVA. The fish freshness monitoring using film indicator was conducted at chilling temperature. The color of colorimetric film became yellow until 8<sup>th</sup> days. Thus, it can be concluded this color change corresponded with TVB-N level are increased.

#### 5. Acknowledgement

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