Color Alteration of Encapsulated Beetroot (*Beta vulgaris* L.) Extract Upon Dissolving in Various pH Treatment

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**Abstract**

It has been known that most natural pigments are unstable upon exposure against several environmental factors, such as thermal treatment, light, oxidizing or reducing agents, as well as acid or alkaline compounds. Encapsulation procedures are often adopted to adjust the solubility and provide protection to the natural pigments. Here, we prepared an encapsulated beetroot extract as the candidate of red bio-colorant. The primary aim of the present study is to investigate color alteration of encapsulated beetroot (*Beta vulgaris* L.) extract upon dissolving in various pH adjustment and prolonged storage. The McIlvaine buffer was prepared in various pH range, i.e. from 2 to 11. The encapsulated extract (0.1% w/v) was dissolved and the color of the solutions were measured regularly until 3 days storage under darkness at 20°C. Any degradation or structural changes will cause color alteration, which were monitored through L*, a*, b* values, the hue angle (H°), chroma values (C), as well as color difference (ΔE). The results showed that pH 4 was the most favorable condition that brings the least impact to the color alteration, even when the colored solution was kept in prolonged storage until 9 days.

**INTRODUCTION**

Food color greatly influences consumers preference, selection, and acceptance toward food products [1]. The use of food colorants is inevitable in almost all processed food either to intensify and uniform the color or to give a new color for colorless material [2]. Nowadays, the development of natural colorants is fraught with challenges due to the current dominance of highly processed food as well as the massive use of synthetic colorants. Natural pigments are obviously preferred for their biological benefits and long-term consumption, but they are sensitive to heat, light, and pH [3]. The application of encapsulation and lyophilization technologies have been widely adopted in order to improve the stability of natural pigments as well as protect their biological activities [4, 5].

Betalains is a group of water-soluble pigments biosynthesized by plants through the shikimate pathway from betalamic acid as the precursor. The immonium derivative of betalamic acid is yellow (betaxanthins), whereas the condensation product of betalamic acid with *cyclo-*dihydroxyphenylalanine (*cyclo*-DOPA) has purplish red color (betacyanin) [6, 7]. Red beetroot is the most exploited source of betalains. It is often prepared as salad, curry, being roasted, pickled, or even fermented as kvass. In some baby food products, it is used as natural red colorant [8].

The production and thermal study of several candidates of red biocolorant have been reported in our previous study [9]. Betalains of the red beetroot (*Beta vulgaris* L.) was the prominent candidate since it exhibited the highest tinctorial strength and best stability during thermal treatment. It is expected to be more natural, plant-based, having less allergen,
hence its use may solve the ‘carmine problem’. However, its color stability during storage at ambient temperature in various pH has not been determined yet.

The present investigation is aimed to study the color alteration of encapsulated beetroot extract upon being reconstituted in water-based solution at various pH; (i) the distribution of color values by pH tuning, (ii) chroma and color difference, as well as (iii) color evolution at certain pH which showed favorable stability.

EXPERIMENTAL

Extraction and Encapsulation

Samples of red beetroot were purchased from a local market in Malang, East Java, Indonesia. The fresh roots were peeled, washed, and drained prior to extraction. The slow juicer (Hurom HH-SBF11, USA) was employed to perform cold-extraction without any water addition. The extract was blended with 5% (w/v) maltodextrin (Yishui Dadi Corn Developing Co. Ltd., China, DE 10-12%) by means of Ultra-Turrax (IKA T-18, Germany), then stored at -20°C for 24 hours. Furthermore, the frozen material was lyophilized using a freeze dryer (Labconco-Freezone 2.5 L, USA) under low temperature and vacuum condition (-49°C, 0.04 MPa) for 48 hours until the moisture content was under 10%. The dried extract was then crushed using a mortar and stored at low temperature (-20°C) until further analysis. The headspace in the container was flowed with N₂ gas in order to reduce the presence of oxygen as well as prevent unexpected oxidation during storage.

Preparation of Buffer and pH Treatment

The McIlvaine buffer was prepared according to Molina et al. [10] with slight modification in the proportion of citric acid and Na₂HPO₄. The citric acid (< 2%), Na₂HPO₄ (< 2%) and sodium azide (0.02%) were dissolved in filtered aquadest. A water purifier (Thermo Scientific 7133, USA) was used to filter the aquadest before use. Then, the encapsulated beetroot extract (0.1 % w/v) was diluted in buffer solution at pH range between 2 to 11. The storage study was conducted by means of a climate chamber unit (Memmert ICH110, Germany) which was set on 20°C and RH 15.1% without any illumination. The color observation was conducted at day 0 and 3 for all pH values and continued until day 6 and 9 for pH 4 and 8.

CIELAB Color Measurement

The measurement of color value was performed by using a ColorFlex EZ 0530 (HunterLab, USA) according to CIELAB system to quantify the L*(brightness), a* (redness) and b* ( yellowness) of the samples. The chroma (C*), hue (H°), and color difference (∆E) were calculated according to the following formulas:

\[ C^* = \sqrt{a^{*2} + b^{*2}} \]  
\[ H^\circ = \arctan \left( \frac{b^*}{a^*} \right) \]  
\[ \Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \]

RESULTS AND DISCUSSION

Distribution of Color Values by pH Tuning

In the present work, beetroot extract was encapsulated with maltodextrin as the wall materials, and subsequently lyophilized at low temperature and pressure in order to maintain its color as well as the bioactive compounds. The encapsulated extract of beetroot was successfully prepared as vivid red powder, which is ready to be used as natural colorant. The powder showed favourable flowability characteristic as long as it is kept at low humidity (RH 15-30%) and temperature (below 0°C), whereas at room temperature it tends to agglomerate. According to the previous studies [12-14], the hygroscopicity of the encapsulated powder was determined by its sugar content, type of coating agent, as well as the drying method. The high sugar content of beetroot (~10%) [15] and the use of maltodextrin was supposed to mainly contribute to the hygroscopicity of the resulted encapsulated extract.
When the powder (0.1% w/v) was dissolved in buffer at various pH, the color of the solution was varied from red to purplish-red. The distribution of color value at pH 2-11 was shown in Figure 1. Being different from anthocyanins group, the betalains have more homogenous color at a wider range of pH. Among the L*, a*, and b* values, the obvious difference was seen in the a* (redness) value, in which higher a* scores were measured in the lower pH, that is between pH 2-8. As mentioned by Slimen et al. [16], the isolated betalains are stable at a broad pH ranging from 3 to 7, but they will be degraded when the environment is below pH 2 or above pH 9. This degradation is often indicated by the shift of the UV-Vis spectrum and production of betalamic acid (data not shown). Meanwhile, the results of the present study revealed that simple juicing method of beetroot as well as the lyophilization could provide strong red coloration at pH 2-8, and the presence of non-betalains fractions in the beetroot juice as well as the encapsulating material do not alter the intrinsic properties of betalains.

Furthermore, after three days of dark storage, there was a significant reduction in the redness (a*) scores, along with a slight increase in lightness (L*) and yellowness (b*) particularly in alkaline pH. Notable change in color appearance was visible in pH 5 and 6, in which the solution was turned into yellowish red. The buffer solution at pH 4 showed good color stability since it redness value was the highest after three days of storage. The color evolution of encapsulated extract in this acidic environment will be further discussed in the stability study. The elevation of lightness scores was the consequence of the formation of degradation product of betacyanin. Antigo et al. [17] and Herbach et al. [18] reported that degradation of betanin was indicated by the dominance of yellow color, leaning to the increase of lightness and yellowness scores. The degradation pathways of betanin was shown in Figure 2.

Even though the redness score could be simply used to predict the best environment for the beetroot extract, it is also important to evaluate their hue angles. The discrepancy of hue angles was depicted in Figure 3. Based on the position of the hue angles, the least shifts were found at pH 2, 3, and 4, whereas major displacements appeared at pH 9, 10, and 11. After being stored for 3 days, the hue angle at pH 2, 3, and 4 was 340.6°, 342°, and 341°, respectively. This angle indicated a red color of the beetroot extract. On the other hand, that of pH 5 and 9 showed the color evolution into yellowish solution, having H° at 42° an 57°, respectively. The hue angles of the colored buffer solution at pH 6 to 8 were turned into vivid red (from 1° to 11°). Those at pH 10 and 11 were rapidly shifted from purplish-red (322° - 333°) into greenish-yellow (100° - 108°) in one day, and then sluggishly shifted after three days observation (107° - 108°). The previous reports [18-20] have noted that the major fraction in betalains of red beetroot was betacyanin, contributing in the red to purplish-red color, and exhibiting good stability at pH 3 to 7.

**Chroma and Color Difference**

Chroma value was often calculated in order to quantify the strength or dominance of the hue. The graph in Figure 4 shows the alteration of chroma value during dark storage, as the consequence of pH variation. The smallest change of chroma value was observed at pH 4, being consistent to the highest redness score and small shifting of its hue angle after three days dark storage at 20°C.
Despite the fact that alkaline environment also caused low impact on chromatic change, the lightness scores were elevated, revealing the color fading that occurred during storage. In addition, the reflectance spectra pointed the comparable trend (data were not shown). The degradation of reflectance percentage at acidic and neutral state was fall off by 10% to 48%. However, at pH 10 and 11, the extract was extremely degraded by 90% to 92%.

Moreover, the parameter of color difference ($\Delta E$) has been adopted by some practitioners to predict the ability of the consumers in detecting the color change. The calculated data of color differences were given in Table 1. The validated value compromises $\Delta E < 1$ for a normally invisible difference, $1 < \Delta E < 2$ for a very small difference that is only obvious to a trained eye, $2 < \Delta E < 3.5$ for medium difference that is obvious to an untrained eye, and $\Delta E > 3.5$ for an obvious difference [21]. In fact, the calculated data of color differences were all greater than the limit of visible difference that could be detected by normal eyes. However, the significant small color difference was obtained in colored buffer solution which was adjusted at pH 4.

The comparable pattern was observed in the thermal study of encapsulated beetroot extract which was previously reported [6]. After thermal treatment (60°C, 30 minutes), color differences of the colored buffer solution were gradually decreased from both pH 2 and pH 11 into the lowest point at pH 4. Nevertheless, in the present study, lowered $\Delta E$ value at pH 11 after three days dark storage at 20°C somehow belonged to an anomaly. The thermal study of the encapsulated extract of red spinach as well as dragon fruit also gave a similar downward trend at pH 11.

Figure 3. Effect of pH on the hue angle of the encapsulated beetroot extract after being dissolved in buffer solution on the first day and after 3 days under dark storage at 20°C. The black and red dots represent the hue angle on the first day and after storage, respectively.

Figure 4. The alteration of chroma value of the encapsulated beetroot extract after being dissolved in buffer solution and stored for 3 days under darkness at 20°C, as the result of pH variation.
Table 1. Color differences of encapsulated beetroot extract after being dissolved in buffer solution, as the consequence of 3 days storage (dark, 20°C) in various pH.

<table>
<thead>
<tr>
<th>pH value</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11.46 ± 0.94</td>
</tr>
<tr>
<td>3</td>
<td>15.24 ± 1.59</td>
</tr>
<tr>
<td>4</td>
<td>6.37 ± 0.24</td>
</tr>
<tr>
<td>5</td>
<td>33.70 ± 0.31</td>
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<tr>
<td>6</td>
<td>17.91 ± 2.51</td>
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<tr>
<td>7</td>
<td>24.47 ± 0.19</td>
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<tr>
<td>8</td>
<td>25.26 ± 1.56</td>
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<tr>
<td>9</td>
<td>28.89 ± 0.79</td>
</tr>
<tr>
<td>10</td>
<td>40.11 ± 0.28</td>
</tr>
<tr>
<td>11</td>
<td>23.48 ± 3.58</td>
</tr>
</tbody>
</table>

Color Evolution at pH 4 and 8

In order to investigate color evolution of the encapsulated beetroot extract in prolonged storage, the buffered solution with pH 4 and 8 were determined as representatives. Figure 5 below shows the percentage of decrease in redness (a*) scores of the encapsulated beetroot extract after being dissolved in buffer solution pH 4 and 8, and then stored for 9 days under darkness at 20°C.

CONCLUSIONS

Color alteration was detected after the encapsulated beetroot extract was dissolved in a buffered solution at various pH, from 2 until 11, and stored under dark storage at 20°C. The red hue was resulted at acidic to neutral environment, whereas the alkaline environment caused purplish-red appearances. The solution with pH 4 was found as the most favorable condition to slow down its color evolution, as indicated by slowest redness change, closest hue displacement, and lowest color difference during dark storage up to 9 days at 20°C. Further observation upon its application in complex food system must be carried out.

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REFERENCES

Telah diketahui bahwa pigmen alami bersifat sangat rentan terhadap pengaruh lingkungan sekitarnya, seperti perlakuan panas, cahaya, senyawa oksidator-reduktor, serta suasana pH asam atau basa. Prosedur enkapsulasi seringkali digunakan untuk mengatur kelarutan pigmen alami dan juga memberikan perlindungan pada molekul pigmen. Dalam penelitian ini, ekstrak umbi bit dienkapsulasi dan dapat digunakan sebagai kandidat pewarna alami merah. Tujuan utama penelitian ini adalah untuk menyelidiki perubahan warna yang terjadi pada enkapsulat ekstrak bit yang dilarutkan pada buffer McIlvaine dengan variasi pH 2 hingga 11. Serbuk ekstrak bit terenkapsulasi dilarutkan pada konsentrasi 0.1% (b/v) dan warna larutan diukur sebelum dan selama penyimpanan gelap pada suhu 20°C. Adanya degradasi dan atau perubahan struktur akan menyebabkan perubahan warna, yang dapat dideteksi melalui nilai L*, a*, dan b*, Hue, Kroma, serta nilai perbedaan warna (ΔE). Hasil penelitian menunjukkan bahwa pH 4 merupakan kondisi yang dapat mempertahankan stabilitas pigmen pada ekstrak bit selama penyimpanan gelap, bahkan hingga 9 hari.

Kata kunci: umbi bit, warna, ekstrak, enkapsulasi, stabilitas pH