Effects of pH and Storage Time on the Stability of Papaya and Carrot Extracts

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Abstract
Papaya and carrot are rich in natural pigments, especially β-carotene. Unfortunately, in most cases, natural pigments are less stable than the synthetic ones when they are put in a variety of treatment conditions, such as pH. In this work, the stability of the papaya and carrot extracts were evaluated in various pH, which ranges of 1–10 for 7 days. The extracts were obtained by a slow juicer, followed by encapsulation and drying by a freeze dryer. The extracts were then dispersed in buffer solutions and the chroma and color difference (ΔE) values were calculated from the obtained lightness (L*), redness (a*), and yellowness (b*). The results showed that carrot extract was better than the papaya extract in terms of chroma values. It was also shown that both extracts were not stable under acidic condition, but more stable in neutral to alkaline condition. The acid condition facilitated the cleavage of the conjugated bonds in the β-carotene, which in turn caused the color degradation in the papaya and carrot extracts.

INTRODUCTION
Natural pigments have many good benefits for our health [1-3]. Furthermore, they are abundantly found in nature and are not harmful to the environment. Therefore, natural pigments are widely applied in various fields, such as for the coloring of food, cosmetics, and pharmaceuticals [4-6]. Unfortunately, in general, natural pigments are less stable in a variety of treatment conditions such as pH, temperature, and light, as compared to the synthetic pigment [7-9].

One approach to stabilizing the natural pigments is by encapsulation, which can be defined as a method of packaging a substance into a small size with the help of coating material [10]. The method that was applied in this study was freeze drying [11-13]. The use of freeze-drying is suggested since it can avoid the use of high temperature, which is one of the factors that lead to the instability of the natural pigments.

In this study, the stability of the natural pigments extracted from papaya and carrot was investigated by varying the pH and storage time for several days. Papaya and carrot have been known as the good source of carotenoid, especially β-carotene [14-16]. As a provitamin, β-carotene has many functions in the human body, such as for growth and development purpose, for the maintenance of the immune system and good vision. Therefore, the study on the stability of the pigments extracted from papaya and carrot is important. This study demonstrated that the stability of the extracted natural pigments from papaya and carrot was influenced by the pH condition.

EXPERIMENTAL
General
The main raw materials that were used in this study were papaya and carrot fruits, freshly purchased from a supermarket in Malang, East Java, Indonesia. The coating agent that was used in the encapsulation process was maltodextrin with a dextrose equivalent (DE) of 10–12% (Yishui Dadi Corn Developing Co., Ltd.). In order to prepare various buffer solutions with pH of 1–10, several chemicals were used following the reported literature [17]. Potassium chloride, KCl (Merck, 99%) and hydrochloric acid, HCl (Merck, 37%) were used to prepare a buffer solution with pH of 1 and 2. Citric acid, C6H8O7 (Chameleon, 99%) and trisodium citrate, Na3C6H5O7 (Chameleon, 99%) were used to prepare the buffer solution with pH of 3, 4, and 5. Buffer solutions with pH of 6,7, and 8 were prepared using certain volumes of potassium dihydrogen phosphate, KH2PO4 (Chameleon, 99%) and sodium hydroxide, NaOH (Chameleon, 96%). Sodium tetraborate decahydrate or...
brom, Na₂B₄O₇·10H₂O (Wako, 99%) and HCl were used to prepare the buffer solution with pH of 9, while borax and NaOH were used to prepare the buffer solution with pH of 10.

The extraction of papaya and carrots was carried out by a HUROM slow juicer (HH-SBF11). A freeze dryer (Labconco 220) was used during the encapsulation process. A ColorFlex EZ spectrophotometer (HunterLab) was used to study the stability of the extracts.

Extraction of Papaya and Carrot Fruits

The papaya and carrot fruits were washed and peeled first, then cut into smaller pieces. The small pieces of papaya and carrot were then put into a slow juicer to obtain the papaya extract and carrot extract, respectively. The slow juicer was used here to reduce heat and friction, which could destroy the pigment. Water was not added during the extraction, and thus, the water content in the extract only came from the fruit.

Encapsulation of Papaya and Carrot Extracts

The encapsulation of papaya and carrot extracts were carried out with the help of maltodextrin as the encapsulating agent, followed by the freeze-drying process and the storage of the freeze dried-extract. In the encapsulation process, the required amount of maltodextrin was determined first so that the ratio of the total weight of solid to the solution was ca. 0.1–0.2%. The total weight solid was obtained from the weight of maltodextrin and extract solid, while the weight of the solution came from total water including the water content in the extract. The mixture of maltodextrin, water, and fruit extract was then homogenized well. The mixture was stored in a freezer for overnight to avoid any thermal degradation before it went for the next procedure, which was the drying process. The frozen mixture containing maltodextrin, extract, and water, was then further dried by the freeze dryer for 48 h at a temperature of -45 °C and a pressure of 0.02 MPa. This process was required to remove the water content. The obtained dried encapsulated papaya and carrot extracts were then ground. They were stored in the freezer and put in the container which was flown by nitrogen and free of air to avoid any thermal degradation and oxidation reactions, respectively.

Stability Test at Various pH and Storage Time

In order to study the effect of pH on the stability of the papaya and carrot extracts, buffer solutions with the pH range of 1–10 were prepared in a similar way to the reported literature [17]. In a typical stability test, 0.375 g of encapsulated extract was added in 25 mL of a buffer solution with a certain pH. In order to have a good dispersion, each mixture was mixed well before they were put in the climate chamber. The stability tests were carried out for 0–7 days under the dark condition. The conditions in the climate chamber were made constant at the temperature of 25 °C and relative humidity (RH) of 15%. The stability of the pigment extract was evaluated by a color measurement on a ColorFlex EZ spectrophotometer. The lightness (L*), redness (a*) and yellowness (b*) values of each extract at each pH condition was measured after 0, 1, 2, 4, and 7 days where the monitored wavelength ranged from 400 to 700 nm. Each measurement was repeated three times and the average value was taken into the calculation. The stability of the extracts could be then evaluated from the stability of its chroma values and color difference (ΔE). Chroma and ΔE values were calculated from Equations (1) and (2), respectively.

\[
\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)
\]

\[
\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)
\]

RESULTS AND DISCUSSION

Papaya Extract

The encapsulated papaya extract showed a yellow-orange color. As shown in Figure 1, in general, the papaya extract could not be dispersed well in the buffer solution with pH of 1–10. This would be due to the fact that the β-carotene could not be dissolved completely in water [18]. The use of various pH did not give different color appearances in the extract. In order to study the stability of the papaya extract in various pH for several days, the chroma values were calculated from a* and b* values. Chroma value is a level of color strength.

Changes in the chroma values during seven days of storage are shown in Figure 2. It can be seen that in the initial point, the chroma values were varied with the changes in the pH. However, there was no clear trend could be observed within the pH range. The lowest chroma value was 5.5, which was observed at the pH of 2, while the highest chroma value was 10.7, which was observed at the pH of 10. It was worthy to note that the high chroma value of 9.6 was also observed at the pH of 7. These results showed that the color level of the papaya extract was strong at pH of neutral to alkaline condition, while under the acidic condition the color level was weak. Unfortunately, after storing the extract in the buffer solution for several days, the chroma values of papaya extract decreased in most pH conditions, suggesting that the color degradation occurred.

Figure 1. The appearance of encapsulated papaya extract in a buffer solution with pH of 1–10 at a storage temperature of 25 °C and dark condition after (a) 0, (b) 1, (c) 2, (d) 4, and (e) 7 days.
In order to see the correlation between the color change and the storage time at a certain pH condition, the color difference (ΔE) was calculated. The calculated ΔE values were then plotted against the storage time and the correlation for each pH condition was determined. The correlation between the two values can be considered strong if the correlation coefficient \((r)\) is more than 0.7. If the value is below 0.7, the correlation is considered moderate \((0.5 < r < 0.7)\) or weak \((0.3 < r < 0.5)\). In addition to the \(r\) value, the gradient value also gives important information. The low gradient suggests that the difference of the ΔE over the storage time is small, showing the high stability of the pigment over the storage time. On the other hand, the high gradient value shows the large difference of the ΔE over the storage time, indicating the color degradation or the low stability of the extract. Based on these definitions, in order to determine the best pH condition that gives high stability of the extract, both the \(r\) and gradient values shall be evaluated.

As listed in Table 1, most pH gave a strong correlation between the ΔE and the storage time, except for the pH of 6 and 8. Special attention was given to the pH that showed a very high correlation close to 0.9 or more, which pH values were 2, 3, 4, 7, and 9. Among these pH, the gradient values were in the order of pH 7 < 9 < 2 < 3 < 4. This result suggested that the lowest ratio of the color difference to storage time was achieved at pH 7, followed by pH 9. While the pH of 7 was the optimum pH condition to preserve the stability of the papaya extract, the pH of 9 was also suitable to maintain the stability of the papaya extract.

**Carrot Extract**

Compared to the encapsulated papaya extract, the carrot extracts gave a stronger color appearance of red-orange than the papaya extract with the yellow-orange color. As depicted in Figure 3, after the dispersion of the extract in various buffer solutions with pH of 1–10, initially the extract was dispersed well, but after some time they were separated from the solution.

**Table 1. Correlation of ΔE values and storage time of encapsulated papaya extract in a buffer solution with various pH**

| pH  | Correlation Coefficient \((r)\) | Equation               | Gradient |
|-----|---------------------------------|------------------------|----------|
| 1   | 0.7323                          | \(y = 0.0966x + 4.8237\) | 0.0966   |
| 2   | 0.9850                          | \(y = 0.1983x + 0.4177\) | 0.1983   |
| 3   | 0.9952                          | \(y = 0.4376x - 0.2553\) | 0.4376   |
| 4   | 0.9557                          | \(y = 0.5476x + 0.0902\) | 0.5476   |
| 5   | 0.7325                          | \(y = 0.0830x + 2.5610\) | 0.0830   |
| 6   | 0.6173                          | \(y = 0.1011x + 3.0365\) | 0.1011   |
| 7   | 0.9924                          | \(y = 0.0956x + 3.5103\) | 0.0956   |
| 8   | 0.0707                          | \(y = 0.0104x + 3.2788\) | 0.0104   |
| 9   | 0.8822                          | \(y = 0.1475x + 2.4783\) | 0.1475   |
| 10  | 0.8408                          | \(y = 0.2754x + 4.9655\) | 0.2754   |

**Figure 2.** Chroma values of encapsulated papaya extract stored in a buffer solution with pH of 1–10 at 25°C and dark condition for 7 days.

**Figure 3.** The appearance of encapsulated carrot extract in a buffer solution with pH of 1–10 at a storage temperature of 25°C and dark condition after (a) 0, (b) 1, (c) 2, (d) 4, and (e) 7 days.

As shown in Figure 4, at the initial time the chroma values of the carrot extract were in the range of 14.5–20.9, which were much higher than those observed in the papaya extract. This is in good agreement with the stronger color appearance of the carrot extract. The lowest or highest chroma value was obtained when the solution pH was 1 or 7, respectively. These results showed that the highest level of carrot extract color can be obtained under the neutral condition, while under acidic condition, the color level was weak, similar to the case of papaya extract shown previously. After storing in the buffer solution for several days, even though the chroma values of the carrot extract could be maintained better than the papaya extract, the decrease in the chroma values could not be avoided. This result clearly showed that the stability of the pigment in the carrot extract was affected by both pH and storage time.
The effect of the storage time to the stability of the extract was investigated by analyzing the ∆E values obtained at each the storage time. Table 2 shows the correlation of ∆E values and storage time of the encapsulated carrot extract in a buffer solution with various pH. Based on the linear equation between the ∆E values and the storage time, the positive correlation could only be observed at pH of 1 and 9. Compared to pH 1, pH 9 gave lower gradient value, suggesting that pH 9 would be better to preserve the color stability of the carrot extract than the pH 1. This result again demonstrated that the alkaline condition is more suitable for storing the carrot extract than the acidic condition.

Table 2. Correlation of ∆E values and storage time of encapsulated papaya extract in a buffer solution with various pH

| pH  | Correlation Coefficient (r) | Equation | Gradient |
|-----|----------------------------|----------|----------|
| 1   | 0.9788                     | y = 0.6816x − 0.9020 | 0.6816   |
| 2   | 0.9775                     | y = 0.0157x + 4.8816  | 0.0157   |
| 3   | 0.7776                     | y = 0.6364x + 1.2740  | 0.6364   |
| 4   | 0.5328                     | y = -0.2191x + 5.3216 | -0.2191  |
| 5   | 0.3523                     | y = 0.0999x + 3.3489  | 0.0999   |
| 6   | 0.1004                     | y = 0.4760x + 2.9808  | 0.4760   |
| 7   | 0.1393                     | y = 0.1084x + 5.2524  | 0.1084   |
| 8   | 0.8139                     | y = -0.3857x + 4.4915 | -0.3857  |
| 9   | 0.9521                     | y = 0.4581x + 1.3943  | 0.4581   |
| 10  | 0.3720                     | y = 0.3023x + 1.4528  | 0.3023   |

Effect of pH on the Color Stability

As aforementioned above, the chroma values of both the papaya and carrot extracts were more decreased when the extracts were put under acidic condition than under neutral and alkaline conditions. Therefore, it could be suggested that the papaya and carrot extracts shall be stored in neutral to alkaline conditions, such as pH of 7–9. It has been generally accepted that the color of pigment would depend on the conjugated bonds in the pigment itself. Stronger color intensity is expected when the pigment has more conjugated bonds. Since β-carotene is one of the main pigments found in papaya and carrot fruits, the effect of pH on the color stability of the papaya and carrot extracts is discussed relating to the β-carotene.

The oxidation reaction can occur when the β-carotene pigment is put under acidic condition [19,20]. When the condition is acidic, the H⁺ from the buffer solution can attack the conjugated bonds on the structure of β-carotene, resulting in a decrease in color intensity. It has been suggested that under acidic condition, a neutral carotene molecule would form a carotenoid carbocation as shown in the Equation (3). Moreover, the degradation of some carotenoids was reported to occur in the presence of acid [21].

\[ \text{Car} + \text{AH} \leftrightarrow (\text{CarH}^+...\text{A}) \leftrightarrow \text{CarH}^+ + \text{A}^- \]  

**CONCLUSION**

Papaya and carrots could be extracted and encapsulated by a freeze-drying method. Compared to the papaya extract, the carrot extract gave higher chroma values under all pH conditions. However, the high chroma values could be observed when the extract was put under neutral or alkaline conditions. For both extracts, the lowest chroma values were observed when the buffer solution was acidic. In general, both extracts were not stable after several days, especially when they were stored in acidic condition. It was shown that the papaya and carrot extracts were more stable when they were stored in neutral to alkaline conditions.

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**REFERENCES**

[1] Melder, H. M., Holven, K. B., Holvik, K., and Latvik, M., Risk assessment of beta-carotene in food supplements. Scientific Opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy, Norwegian Scientific Committee for Food Safety (VKM) Report, 2015, ISBN: 978-82-8259-155-3, Oslo, Norway.

[2] Leong, H. Y., Show, P. L., Lim, M. H., Ooi, C. W., and Ling, T. C., Natural red pigments from plants and their health benefits – A review Food Rev. Int. 2017, 34(5), 463–482, doi: 10.1080/87595129.2017.1326935.

[3] Wootton-Beard, P. C., and Ryan, L., Improving public health?: The role of antioxidant-rich fruit and vegetable beverages, Food. Res. Int., 2011, 3135–3148, doi: 10.1016/j.foodres.2011.09.015.

[4] Arad, S. M, and Yaron, A., Natural pigments from red microalgae for use in foods and cosmetics, Trends Food. Sci. Technol., 1992, 3, 92–97, doi: 10.1016/0922-2449(92)90145-M.

[5] Aberoumand, A., A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry, World J. Dairy Food. Sci., 2011, 6(1), 71–78.

[6] Sigurdson, G. T., Tang, P., and Giusti, M. M., Natural colorants: Food colorants from natural sources, Annu. Rev. Food Sci. Technol., 2017, 8, 12.1–12.20, doi: 10.1146/annurev-food-030216-025923.

[7] Delgado-Vargas, F., Jiménez, A. R., and Paredes-López, O., Natural pigments: Carotenoids, anthocyanins, and betalains – characteristics, biosynthesis, processing, and stability, Crit. Rev. Food Sci. Nutr., 2000, 40(3), 173–289, doi: 10.1080/10408609917189257.

[8] Koca, N., Karadeniz, F., and Burduliu, H. S., Effect of pH on chlorophyll degradation and color loss in blanched green peas, Food Chem., 2006, 100(2), 609–615, doi: 10.1016/j.foodchem.2005.09.079.
Abstrak
Pepaya dan wortel kaya akan pewarna alami, khususnya \( \beta \)-karoten. Sayangnya, dalam banyak hal pewarna alami kurang stabil dibandingkan dengan pewarna buatan ketika ditempatkan di bawah berbagai kondisi, seperti perlakuan pH. Dalam penelitian ini, stabilitas ekstrak pepaya dan wortel dievaluasi menggunakan berbagai pH yang berkisar 1-10 selama 7 hari. Ekstrak diperoxide dengan menggunakan alat pembuat jus lambat, diikuti oleh enkapsulasi dan pengeringan dengan menggunakan pengering beku. Ekstrak kemudian didispersikan dalam larutan penyegar dan nilai kroma serta perbedaan warna \( \Delta E \) dihitung dari tingkat kecerahan, warna kemerahan, dan warna kekuningan. Hasil penelitian menunjukkan bahwa ekstrak wortel lebih baik dibandingkan ekstrak pepaya dalam hal nilai kromanya. Juga ditunjukkan bahwa kedua ekstrak tidak stabil dalam kondisi asam, tetapi lebih stabil pada kondisi netral hingga basa. Kondisi asam memfasilitasi pemutusan ikatan terkonjugasi pada \( \beta \)-karoten, yang selanjutnya menyebabkan degradasi warna pada ekstrak pepaya dan wortel.

Kata kunci: pH, waktu penyimpanan, ekstrak wortel, ekstrak pepaya, stabilitas