Time Temperature Indicator Label using Black Corn Extract and Chitosan Matrix

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Abstract. Time-temperature indicator (TTI) labels have been widely researched and some have been used commercially due to their ability to provide food product quality information through changes in label color when exposed to temperature over a specified period of time. The TTI label consists of functional dyes and matrices. The purpose of this study was to develop a TTI label that uses natural dyes derived from black corn extract and chitosan as the matrix. Labels were prepared using a simultaneous method then casted on top of PET substrate. Absorption properties of the black corn extract was investigated by using UV-Vis Spectrophotometer. The label colors were characterized using digital photography and analyzed with CIELab color code. The color of the labels were observed over time at temperature conditions of 10, 25 and 40 °C. The extract solution is red at pH 2-3, then pink at pH 4-5, purple at pH 6-7, violet at pH 8, and turned to yellow at pH 9-13 which indicates that the extract contains anthocyanin. Due to time period, the label changes color from purple to blue to yellow, with the fastest color changes occurred at higher temperature of 40 °C and slowest at lower temperature of 10 °C. The shelf life test on different RH conditions shows that the label stored at RH 33% is more stable than at higher RH of 76%. The labels show irreversible properties which is one of the requirements as a time temperature indicator label. These results indicate that the label of black corn extract with chitosan has the potential to be applied on smart packaging.

Keywords: TTI labels; black corn; chitosan; anthocyanin

1. Introduction

Time-temperature indicator (TTI) label is a device embedded in a product package that is able to provide product quality information due to exposure to temperature over a specified period of time. A good TTI required the ability to indicate a clear and continuous irreversible reaction to temperature changes [1]. Studies on TTI label development have been carried out, such as the study of Pereira et al. [2] who developed TTI based on chitosan polymer and PVA with anthocyanin extract from purple cabbage. Moreover, the type of TTI that is often used commercially is TTI based on enzymes, such as the tyrosinase enzyme to detect the freshness of turbot sashimi [3].

TTI label is composed of functional dye which able to change color to temperature, and matrix as a dye binder. Anthocyanin is a good source of dye for TTI because it is sensitive to environmental
changes so that it provides a wide range of color. Anthocyanin can be found in many plants. In general, several factors influence the stability of anthocyanin such as pH, temperature, light, oxygen, enzymes, metal ions and sugar content [4]. Chitosan is chosen as a matrix because it is biodegradable, non-toxic and easily obtainable because it is a deacetylation form of chitin which is the second most natural polymer in nature after cellulose [5]. Chitosan dissolves in acidic solutions, so that it can be applied in various forms such as solutions, gels, fibers and films. This makes it attractive for use as a dye binding matrix.

In this study, anthocyanin was obtained from black corn extract, while chitosan is used as a matrix. The use of non-toxic ingredients was aimed to make the TTI label safe to apply to packaging products.

2. Materials and Methods

2.1 Preparations of TTI Labels

Black corns were purchased from the local market. The black corn seeds were separated from the stalk then dried in an oven at 40°C for 24 hours. The dried seeds were powdered with food grinder. Solvent used to extract the black corn powder were prepared using 1.5M hydrochloric acid obtained from Mallinckrodt Pharmaceutical (St. Louis, MO, USA) and absolute ethanol from J.T Baker with a volume ratio of 15:85. Black corn powder of 80 g were macerated in 100 ml of the solvent, then stored at 5°C in dark condition for 24 hours. The mixed materials were filtered using Whatman filter paper no. 40. Then its precipitation was further removed using Velocity 14R (Dynamica) centrifuge at a speed of 7500 rpm for 15 minutes. The black corn extract was stored at 5°C in dark condition until use.

Chitosan used as a matrix is a pharmaceutical grade with degree of deacetylation of 92% was obtained from CV. Bio Chitosan Indonesia. Chitosan solution was made by dissolving 2 g of chitosan powder in 100 ml of acetic acid 1% (v/v) which was homogenized with magnetic stirrer at 300 rpm and heated on hot plate of 180°C for 24 hours.

The pH of black corn extract is 0.86 then adjusted to 2.0 with 0.5M of NaOH solution. The extract was mixed to the chitosan solution simultaneously with the ratio of 1:3. The TTI label was prepared by casting the mixture solution of 1 ml on a PET substrate of 2 cm in diameter.

2.2 Characterization of UV-Vis spectrum of anthocyanin from black corn extract

Characterization of the UV-Vis spectrum of the black corn extract was carried out using the UV-Vis Genesys 10S Spectrophotometer from ThermoFisher Scientific. The extract was varied to pH of 2-13.

2.3 Experiment of label color changes against temperature and time

Test on indicator label was done by placing the labels in chambers with fixed temperatures of 10, 25 and 40°C. The label color changes were observed every 3 hours interval. The labels were shot in MiniStudio with illuminance of 4950 lux using Canon EOS 750D camera (ISO 400, Av mode). The image results were further processed using ImageJ color analysis software to obtain color intensity information in RGB color code. The RGB values were then converted to CIELab color code. The L* value indicates lightness that ranges from 0 to 100. The value of a* shows level of greenness (negative) to redness (positive), while b* shows the level of blueness (negative) to yellowness (positive).

2.4 Tests on label stability and reversibility properties

The indicator label stability was tested against relative humidity by placing the label in a chamber with controlled temperature of 25°C and relative humidity of 76%. Another test condition was carried out at temperature of 25°C and relative humidity of 33% setted using MgCl₂ saturated salt. Color changes of the tested labels were observed for 5 days at 1-day interval.

The reversibility properties of the label color changes against temperature were tested by keeping the label in a chamber with controlled temperature. The temperatures were varied from 10°C to 25°C to 40°C, then back to 25°C and finally to the original temperature of 10°C. Each steps were kept in interval
Results and Discussion

3.1 Solution Color and UV-Vis Spectrum of Black Corn Extract due to pH

The color of the black corn extract solution with pH of 2-13 is shown in Figure 1(a). The extract has a wide range of color changes from red at pH 2-3, purplish color at pH 4-7, changes to violet at pH 8, then degrades to yellow at pH 9-13. This color spectrum is common for natural dyes such as anthocyanin and in accordance with the references [4,6].

![Figure 1: Color solution (a) and UV-Vis spectrum (b) of the black corn extract at pH 2-13.](image)

UV-Vis spectrum of black corn extract is shown in Figure 1(b). The maximum absorption at pH 2 and 3 is about 530 nm, which gives a complementary color of reddish purple as seen in Figure 1(a). The dominant anthocyanin structure in these pH is flavylium cation which is stable at low pH and is responsible for the red color seen [4]. At pH 4-7, the structure of anthocyanins is more dominant in the form of carbinol so the purple color looks faded, followed by a decrease in their absorbance values. While at pH 8, quinoidal bases are more dominant, causing the extract to appear bluish. At the UV-Vis spectrum of pH 9-13 it can be seen that the highest absorbance starts from the lowest wavelength of the visible light spectrum, which is 400 nm, and drops dramatically until about 480 nm. This shows that the most absorbed color is blue, resulting in the color of the extract being its complementary which is yellow. At pH 9-13 the most dominant anthocyanin in the extract solution is in the form of chalcone which is responsible for the yellow color. Many studies on anthocyanin sensitivity to pH have been carried out, such as red cabbage [2], sweet potato [6] and Ruellia simplex flower [7] extracts.

3.2 Response of Label Color to Temperature and Time

The TTI label made of the black corn extract and chitosan matrix is shown at Figure 2 (a). The color of the extract before mixed with chitosan was red then changed to purple after mixture. Casting of the solution on PET substrate gives label film with purple color. Response of the label to temperature and time caused the color of the label to change as seen in Figure 2 (b). The labels stored in temperature of 10 and 25 °C show color changes from purple to blue, while in temperature of 40°C the color changed rapidly from purple to blue in 6 hours, and changed to yellow after 360 hours. These results show that the color of the label is affected by temperature conditions and storage time. At temperature of 10°C, the color is more stable to changes compared to higher temperatures of 25 and 40°C.

The color of the label in Figure 2 (b) was characterized using CIE color code to obtain Lab color components. The results were represented in 3-dimensional graph in Figure 3. The lightness (L*) intensity in Figure 3 (a) decreased dramatically through out the first 30 hours of storage time, then reached its minimum value at about 30-60 hours. Afterwards, the L* intensity increased as the storage time increased. Figure 3 (b) shows that the a* intensity is also decreased at the first 30 hours of storage.
time where the color component changes from red to green occurred, then they do not show significant change until the last measurement time of 360 hours. Storage temperature shows no significant effect on both L* and a* intensities. Figure 3 (c) shows that the trend of the b* intensity decreased from the beginning of storage time until the first 30 hours. This means that the blue color component increased during that period. After that, the curve went back up followed by color changes from blue to yellow. This process is affected by the temperature of the storage. The b* intensity is higher when the temperature of the storage is high which is at 40°C. The chromaticity value (ΔE) indicates the total color changes experienced by the label as shown in Figure 3 (d). The chromaticity increased rapidly from 0 to 30 hours at the beginning of storage time. This phenomena corresponds to the trend in L*, a* and b* components as described before. The chromaticity changes give color changes of the labels from purple to blue. At storage time of 360 h, the chromaticity value is about 19 at temperature of 10°C and 25°C, corresponds to the blue color seen on the label. While the chromaticity value increased to 28 at 40°C, corresponds to yellow. This sensitivity to temperature is mostly caused by the sensitivity of the b* intensity to temperature. These results are in agreement with common characteristics of anthocyanin pigments which are sensitive to temperature and storage time [4].

|       | 10 °C | 25 °C | 40 °C |
|-------|-------|-------|-------|
| 0 h   |       |       |       |
| 6 h   |       |       |       |
| 36 h  |       |       |       |
| 360 h |       |       |       |

**Figure 2.** TTI label from black corn extract with chitosan matrix (a) and color of the labels changes to temperature and time (b).
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3.3 Label Stability to Humidity

The TTI label color stability of black corn extract was tested against relative humidity (RH). Based on Figure 4 (a), the TTI labels at RH 33% have higher lightness (L*) value compared to the labels at RH 76%. While the value of a* decreased rapidly in 2 days of storage time corresponds to the decrease of redness intensity toward green color over time as shown in Figure 4 (b). This a* component does not sensitive to RH. In Figure 4 (c) the increasing in blue intensity on the first day occurred more on the labels at RH of 76% than on the labels stored at RH of 33%, indicated by the label value at RH 76% which is more negative compared to the labels at RH 33%. After the first day, the value of b* went back up toward null value which indicates that the yellowness intensity increased. Moreover, the increase in yellow intensity also occurred faster in higher RH of 76% which can be seen by the more positive value of RH 76% compared to RH 33%.

The label from black corn extract and chitosan matrix at RH 76% undergo faster color changes than the labels at RH 33%. In other words, the color of the label is more stable at a lower RH storage condition that is 33%. This is established in the chromaticity graph in Figure 4 (d) which shows that the total color changes of the label at RH of 76% is higher compared to 33%. These results explained that the anthocyanin is affected by water content in the air, where the water molecules can accelerate hydration reaction of the anthocyanin. Therefore, the lower of the water content, the slower the color changes of the label.

![Figure 3](image-url). Changes of color component values of L* (a), a* (b), b* (c) and chromaticity (d) of TTI labels from black corn extract with chitosan matrix to temperature and time.
3.4 Label Reversibility Properties

Irreversibility is an important characteristic required for a TTI label. The results of the reversibility test for the label made of black corn extract and chitosan matrix are shown in Figure 5. The color changes occurred on the label from its original color which is purple to blue on the first day after kept in a temperature of 10°C. As the temperature increased to 25 and 40°C, the label color is still blue after kept in 3 days. When the storage temperature lowered back to 25 and 10°C, the color changed to greenish yellow after 5 days of storage time. The label does not change back to its original color although the temperature returned to 10°C.
Figure 6 shows the analysis of L*, a*, b* and chromaticity of the label in Figure 5. The chromaticity increased from the day-1 until day-3 of storage time as the temperature increased from 10 to 40°C as shown in Figure 6(a). When the storage temperature lowered back to 25 and 10°C on day-4 and day-5, respectively, the chromaticity value did not decrease to its original value as in day-1. From Figure 6(b) it can be seen that the chromaticity value is mostly affected by the irreversibility properties of b* component, while L* and a* components do not show significant changes. The color of the label does not return to its original color even though the temperature is returned to its original state. This irreversible properties of the label shows that the label can record the history of the temperature and time if the label embedded on the intelligent packaging.

4. Conclusion

The label indicator made from black corn extract immobilized on the chitosan matrix has been successfully prepared as a TTI. The color of the label changed from purple to blue to yellow when subjected to certain temperature for a period of time. The higher temperature caused the more color changes compared to lower temperature. The label color is more stable when stored at lower RH compared to higher RH. Moreover, the color change of the label is irreversible. These color changes are mostly influenced by b* component based on the analysis using CIELab color model. This label has a potential to be used to detect product freshness.

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