

Real time on-package freshness indicator for guavas packaging

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Abstract A novel on-package color indicator has been fabricated based on bromophenol blue, and tests have been conducted to assess the freshness of guava (*Psidium guajava* L.). Bromophenol blue (BPB) was immobilized onto bacterial cellulose membrane via absorption method. The BPB/cellulose membrane as color indicator works based on pH decrease as the volatile organic compounds (e.g. acetic acid), produced gradually in the package headspace during developing of guava. Subsequently the color of the indicator will change from blue to green for over-ripe indication, which can be visible to the naked eye. The results showed that the color indicator could be used to determine the state of freshness of the guava at ambient condition (28–30 °C). The color change of the indicators reflects the pH of headspace of the guava packaging. Furthermore, it also in similar trends to the change of several parameters (soluble solids content, texture and sensory evaluation) that normally used to characterize the freshness of guava. Therefore, the indicator can be used for real time visual monitoring of freshness state of packaged guavas.

Keywords Freshness indicator · Bromophenol blue · Guava · Fruit packaging

Introduction

In order to meet the growing demand of consumers for the fresh and safe food, new packaging technologies such as smart packaging, have been developed [1–4]. Smart packaging with intelligent indicator provides information on the integrity and the time–temperature history of the food package, helpful in assuring the quality and safety of the packaged food products [5–7] particularly for perishable fruits and vegetables [8–10].

Once fruits are picked from orchards, the challenge to present fruit in fresh and top condition increases with distance from markets to fulfill more sophisticated consumer demand and need for year-round supply. In the past, loose fruits, often unripe were sold from bins, where it was easily bruised, squeezed and prodded to determine its ripeness state. Then came “ready to eat” fruit, bundled and pre-packaged, but in a manner whereby it is still difficult to determine its preferred state of ripeness.

Since, it is rather difficult to know when the fruit has been reached their preferred state of ripeness, this condition become a barrier to purchase for frustrated consumers. One company from New Zealand produced *ripeSense*TM [11] to eliminate this problem by using an indicator label that reacts to the aromas released by fruit as it ripens. The indicator is initially red in color and gradually changes to orange and finally turns yellow. By viewing the color of the indicator, consumers are able to choose fruit which is at their preferred ripeness. Damage and shrinkage can be reduced as this indicator significantly reduces damage by consumers as they inspect fruit before purchase; and the recyclable indicator pack provides improved hygiene security. This indicator can be applied for pears, kiwifruit, melon, mango, avocado, and other stone fruits [11].

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The number of publications on-package indicators for freshness and ripeness of fruits are still limited. However, some indicators for ripeness detection have been constructed based on ethylene emitted during ripening stage that can be used as a marker for ripeness of fruit, e.g. apple [12, 13]. These indicators are based on molybdenum (Mo) chromophores and change their color from white to blue after reaction with ethylene resulting in the reduction of Mo(VI) to Mo(V). The sensitivity of this indicator can be varied by composition and pH values (pH 1.4–1.5) of the ammonium molybdate solution used. The indicator can be combined with objective color recognition for quantitative measurements of the color change [13]. Other color indicator has been applied for kimchi packaging (fermented vegetable product) to monitor its quality during storage and distribution [14].

Since guava (*Psidium guajava* L.) is a climacteric fruit exhibiting respiratory and ethylene peaks during ripening [15–17]. Along with this development, the fermentative guava metabolites were produced, such as ethanol, acetaldehyde and acetic acid. By enzymatic reaction, alcohol dehydrogenase oxidizes ethanol into acetaldehyde, which is then further oxidized into acetic acid [17]. Therefore, the pH change (the total acidity) occurs due to the production of organic volatile acids (e.g. acetic acid) during ripening state. Thus, as a result of increasing volatile acids, the reduced pH could occur during this development state of guava, where in this case, pH indicator dyes such as BPB (pK_a 3.6), could be used to detect such a pH change during deterioration state of guava.

The purpose of this study was to construct a simple and low-cost on-package color indicator for assessing the freshness state of guavas and their period of salability using BPB/cellulose membrane. Since, currently in Indonesia many supermarkets sold guava packaging, as they sold based on weigh with the price label on the top of the packaging. The BPB/cellulose membrane is highly pH sensitive in the range of pH 3.0–4.6. Color change (from blue to green), as a result of its interactions with pH due to increase in the volatile acids, was monitored directly with color image analysis. Furthermore, the color indicator change was found in similar trend to the change in soluble solids content, texture and sensory evaluation of guava samples. The performance of this color indicator was successfully tested directly for real time visual monitoring of packaged guavas freshness state in ambient condition.

Materials and methods

Chemicals

A stock solution of bromophenol blue (BPB) (Sigma, UK) was prepared by dissolving 10 mg of BPB in 10 ml of ethanol (70 %) to give concentration of 1 mg/ml. All

chemical used were of reagent grade (supplied by Merck, Sigma or Fluka, UK) and used as supplied.

Preparation of bacterial cellulose membrane

As previously described [18], membrane sheets were prepared from bacterial cellulose (10 g), blended until homogeneous, then cast onto the glass plate, pressed and left overnight (12 h). Finally, the white membrane sheet dried at 60 °C and ready to be used.

Preparation of the color indicator labels

Color indicator labels were produced by immersing the membrane sheet into 10 ml of BPB solution (1 mg/ml) at ambient condition (28 ± 2 °C) for overnight (12 h). Practically, the successful immobilization of BPB onto bacterial cellulose membrane was shown by the dark blue color of the membrane as the original membrane color was white. Then, the BPB/cellulose was washed with tap water to remove excessive and unbound BPB solution. Afterward, the membrane was conditioned with acetate buffer (0.01 M) at pH 4.8 (prepared with sodium acetate and acetic acid [19]) to give the dark blue color. The membrane was then dried with an electrical drier (Sanyo, Japan) and cut into the desired shape (Fig. 1).

Packaging of Guava samples

Fresh and ripe whole guavas of normal pH (~ 3.94) purchased at a local fruit garden (at Jember) were used in this study. Three whole guavas with weight around 200 g were used for the analysis, then placed on styrofoam trays (1.05 g/cm^3 , Carrefour, Indonesia) and enclosed into low-density polyethylene plastic film (0.9 g/cm^3 , Carrefour, Indonesia). The samples were stored at ambient condition



Fig. 1 Design of freshness indicator based on BPB/cellulose membrane for guavas packaging with the color indication for fresh (crunchy), medium (firm) and not fresh/overripe (juicy)

(28 ± 2 °C) and normal light exposure as at home or supermarket. While normal guavas (unpacked), were used as reference to separate the effect of packaging (modified atmosphere) on guava. Triplicate packages of the guavas products were sampled at appropriate time intervals (every day after 24 h) to allow for efficient kinetic analysis of pH measurements, SSC, hardeners and skin color index for the study of packaged guavas freshness stored in ambient conditions. All measurements were conducted three times.

Headspace analysis of guava packaging

Ethanol, acetaldehyde and acetic acid concentrations as fermentative guava metabolites were determined by gas chromatography (GC) using static headspace technique as previously described [17] with slight modification as described. Fruit juice was extracted from fruit per replication and stored in a glass vial containing 2 g of NaCl at -40 °C for subsequent analysis. Frozen juice samples were thawed at ambient conditions, and a 5 ml aliquot was transferred to a 15 ml vial and capped with a septum. Samples were incubated at 37 °C for 60 min. A head space sample of 1 ml drawn with a gas-tight syringe was injected into a gas chromatography (GC) (HP 5890, Hewlett Packard, USA) equipped with a packed column (Chromosorb 101) and a flame ionization detector. Nitrogen was used as the carrier gas at flow rate of 30 ml/min, while hydrogen and air were fuel gases having flow rates of 25 and 250 ml/min, respectively. The injector port, column oven, and detector were maintained at 150, 110 and 200 °C, respectively. Qualitative and quantitative determinations of these fermentative metabolites were carried out by comparison with the peak areas of gas samples extracted from aqueous standards of known concentration under similar preparation and GC conditions. The concentration of those guava metabolites were presented in $\mu\text{l/l}$.

Measurement of SSC, pH and texture

The determination of soluble solids content (SSC) was performed by homogenized the guavas (three fruits per replication) and the homogenate filtered through several layers of cheese cloth to obtain clear juice. The SSC (%) was recorded with refractometer (Fisher, Japan). The pH values were recorded by a pH meter (Russel, Moder RL150), with the glass electrode being immersed in the homogenate of guava flesh after the end of the analysis. The texture of guava was measured using texture meter (Rheotex,UK). Each analysis was repeated three times.

Measurement of the color indicator using color analysis

The color indicator was placed inside the package of the guavas samples attached to the plastic wrap of the package

using double transparent tape (3M, US), where the indicator in direct contact with atmosphere inside the package and covered with the label for viewing the color of the indicator along with the reference color for the state of freshness (Fig. 2). Then the samples were stored in ambient condition. Beside, the color indicator can be viewed by the naked eye in term of the color change during guava deterioration state. For quantification of color measurement of the indicator, a simple method was used using a digital camera.

A digital camera (Samsung, ES60, Seoul, Korea) was used to record the color of the indicator with similar set-up, light condition and background for reproducible color value (practically they could be achieved by placing the color indicator in the dark box). The graphics software CorelDrawx4 was then used to analyze the color of the indicator. The term “measure” means that the digital camera is used to obtain the color values of the pixels on the color indicator membrane. The term “analyze” means that CorelDraw is used to measure those color values to obtain color distribution, averages, and so on.

Here, we used the ‘Eyedropper Tool’ for the color value of a selected area in the membrane image of the color indicator. The blue value was used as color value for all membrane color measurements, since the membrane color change from blue to green. By using this method, it is more suitable and simpler as compared to the visual inspection by naked eye as the main purpose for this indicator rather than using reflectance measurement [20]. The principles of color measurement can be found elsewhere [21–23].

Sensory evaluation

In order to describe guava by sensory evaluation, an additional test was performed [24]. At the beginning, each fresh ripe guava was washed with water. Afterward, the



Fig. 2 Application of the freshness indicator for guavas packaging

guavas were stored in a packaged and labeled with the color indicator as described above. Physical and mechanical changes were determined during deterioration state of guava using sensory evaluation, e.g. color, softness, sweetness, juiciness and aroma, and the results were recorded quantitatively based on score from 1 to 5. The measurements were conducted along with the color indicator response. The measurement was done in the laboratory conditions without any special requirements considering the application at shopping center, restaurant, storage room and others. Then, the results of the indicator response were therefore confirmed by the sensory evaluation.

During a period of the investigation (8 days), each day always three of the guava samples were taken from the package, tested and scored by a panel consisting of ten people (four males and six females with age 20–30 years). The grading system was based on scores from 1 to 5 (scale), where score 1 for least dominant and 5 for most dominant for attributes of sweetness, softness, juiciness and aroma.

Subjective rating of skin color of guava was done on a 1–5 scale where: 1, <20 % yellow; 2, 20–40 % yellow; 3, 40–60 % yellow; 4, 60–80 % yellow; 5, >80 % yellow. These color stages were even found useful to define the freshness state in the colored cultivar, fresh ripe guava was used as the background color [17]. In addition, the prevailing subjective status of freshness was assessed from ripening to overripe by classified into fresh (crunchy), medium (firm) and not fresh/overripe (juicy) as shown in the indicator label (Fig. 1). The samples were tasted by individual taster independently, and the mean value of

scores was calculated. Furthermore, the color change of indicator label of the guava sample was investigated every day (after 24 h) during the period of the investigation.

Results and discussion

Ethanol, acetaldehyde and acetic acid

Accumulation of ethanol, acetaldehyde and acetic acid as fermentative guava metabolites were affected by time of storage (Fig. 3). Ethanol concentrations in early stored ripe guava were comparatively more than those at day 3 and day 6. The accelerated guava metabolisms during ripening at ambient conditions might have contributed to the reduction of ethanol from the guava tissue. Acetaldehyde and acetic acid content increased during ripening as compared to early day of storage which was opposite to ethanol (Fig. 3). The accumulation of acetaldehyde in particular during ripening of guava has been reported to contribute both to the removal of astringency and to develop aroma volatiles [17, 26]. In addition, a significant reduction in the pH was observed due to acetic acid formation during ripening state. Ethanol, acetaldehyde contribute significantly to inhibit ripening and flavor quality, but their biosynthesis in excess (increasing acetic acid concentration) leads to the off-flavor development in fruit [29]. Partial anaerobic respiration during modified atmosphere storage, like in this case, might has also resulted in the accumulation of these anaerobiosis metabolites [29, 32]. Subtropical fruits including

Fig. 3 Ethanol, acetaldehyde and acetic acid concentration (ul/l) in guava during storage at ambient conditions

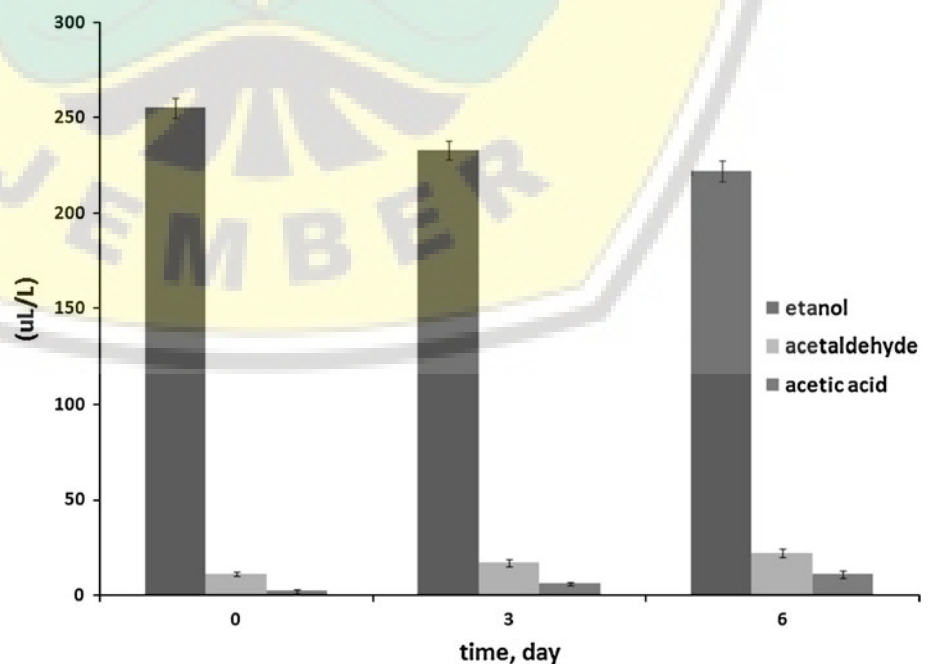


Fig. 4 The rate of color changes of the indicator response towards guava freshness state

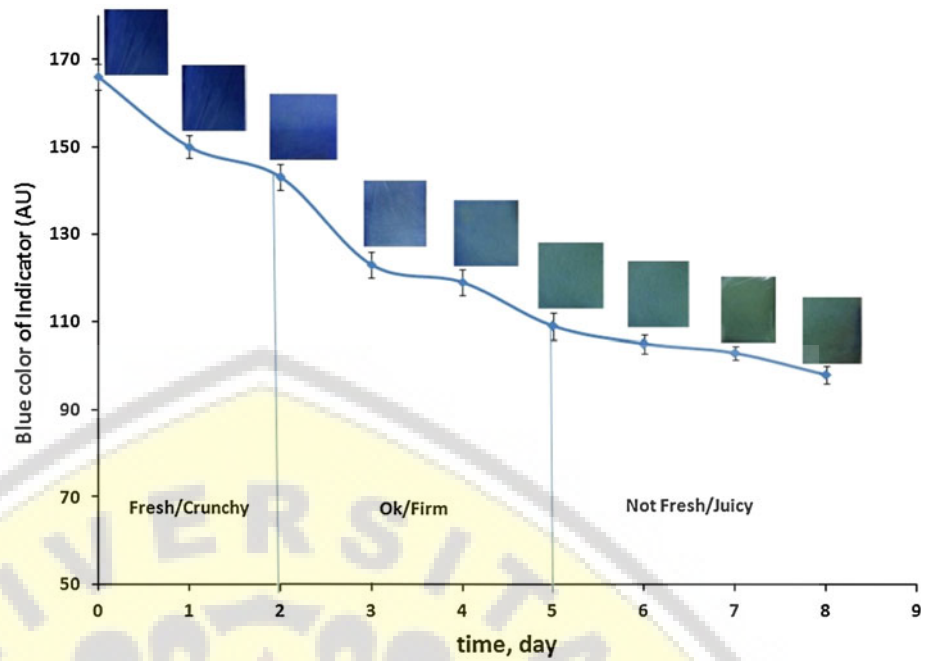
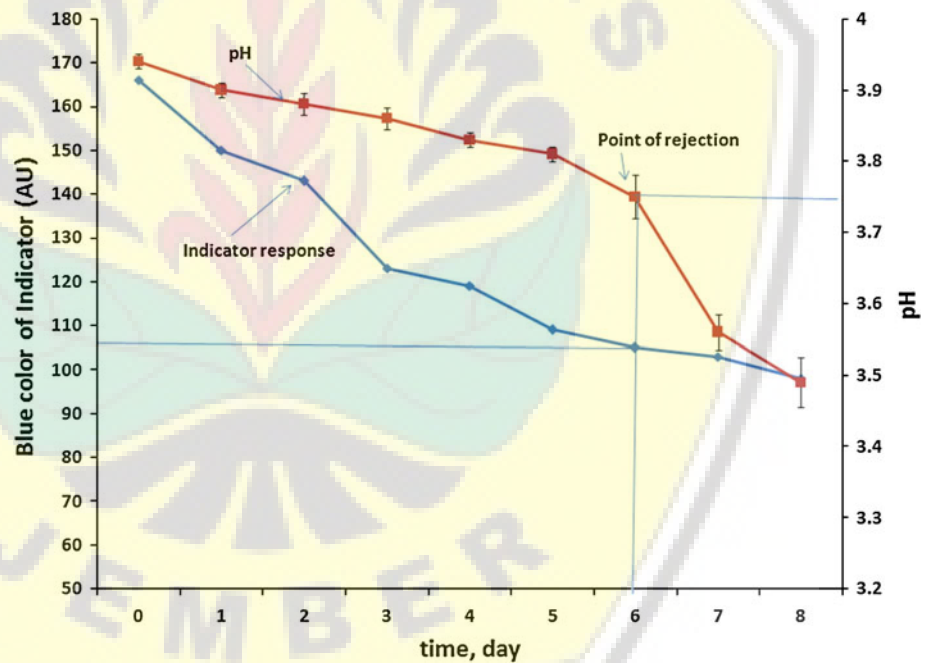


Fig. 5 The pH values of guava samples and the indicator response



guava have been proposed to be among the most sensitive to anaerobiosis damage [29].

Response of the indicator towards freshness of guavas

All color indicators were placed inside the package, in close proximity (2 cm) to the guava samples in order to detect to the pH decrease as the volatile acids released gradually in the package headspace due to guava development during the ripening state, with a very distinct color

change from blue to green. While, the other color indicator was placed outside, on the top of package guava as a blank and expose to the atmospheric air and normal light during the period of the investigation.

The color indicators were monitored periodically until no further color change was observed. While the blank color indicators did not show any color changing during investigation, even they were also exposed to the atmospheric air and normal light. Furthermore, the color labels were stable within 3 months or more, since no color change was

Fig. 6 Correlation between the color indicator towards pH values at 3.56–3.94

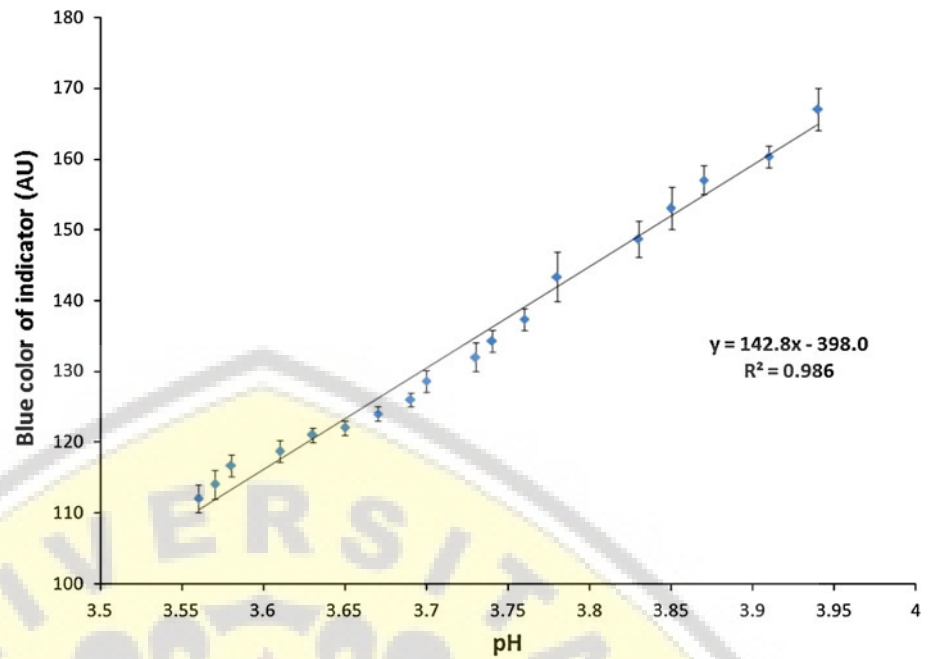
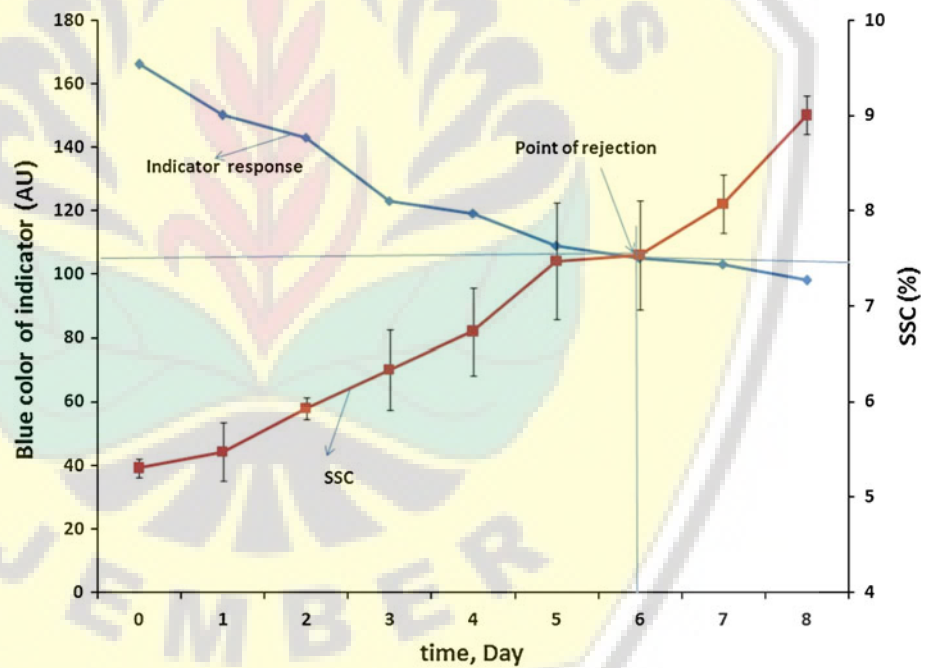


Fig. 7 The SSC values of guava samples and the indicator response



observed during this period of the investigation. Figure 4 shows the rate of color change of the color indicator (intensity of blue in arbitrary unit) towards various freshness states. In Fig. 4, the color indicator response decrease steadily (as the indicator color change to green) within 8 days of the experiment was observed. Here, the color indicator gradually changed color from blue to green after 5 days. Furthermore, visual inspection did not detect any differences in color changes between these indicators of different batch samples. The color indicator response as color change was

similar with deterioration state of the climacteric nature of guava that is well reported in the literature [15–17]. The onset of overripe was detected after 5 days. This indicated that the packaged guava released volatile acids (e.g. acetic acid) at a relatively slow rate in comparison with normal guava, since its freshness lasted longer within 5 days. On the other hand, the packaging influence on other acid compounds in the guava, such as ascorbic acid, was found positive and effective in preventing the losses of this acid in fruit during ripening stage [25, 26].

Fig. 8 The texture values of guava samples and the indicator response

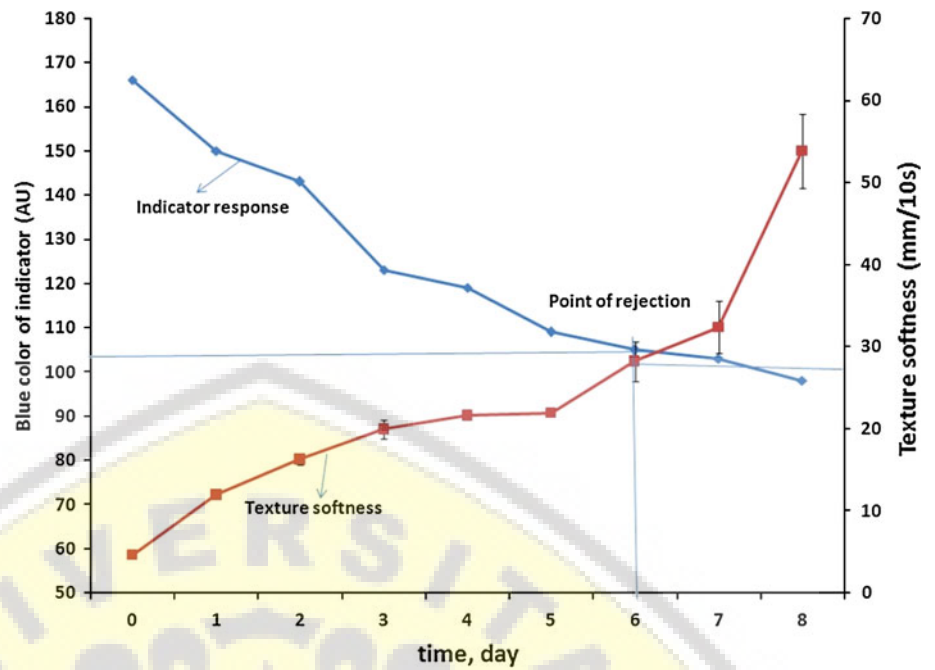


Table 1 The results of the sensory evaluation of the guavas packaging samples

Stored time (day)	Color	Sweetness	Softness	Aroma	Juiciness
0	1.0 ± 0	5.0 ± 0	1.0 ± 0	5 ± 0	1.0 ± 0
1	1.1 ± 0.30	4.9 ± 0.30	1.2 ± 0.4	5 ± 0	1.1 ± 0.3
2	1.9 ± 0.30	3.9 ± 0.30	1.9 ± 0.3	4.6 ± 0.4	2 ± 0
3	2.1 ± 0.30	3.6 ± 0.49	2.3 ± 0.46	3.7 ± 0.46	2.2 ± 0.4
4	3.0 ± 0.45	2.8 ± 0.40	3.2 ± 0.4	3.2 ± 0.4	3.1 ± 0.54
5	3.2 ± 0.40	2.6 ± 0.49	4.2 ± 0.4	2.7 ± 0.46	3.3 ± 0.46
6	3.8 ± 0.40	1.8 ± 0.40	4.4 ± 0.49	2.1 ± 0.3	4.1 ± 0.3
7	4.8 ± 0.40	1.0 ± 0	5.0 ± 0	2.0 ± 0	5.0 ± 0
8	5.0 ± 0	1.0 ± 0	5.0 ± 0	1.0 ± 0	5.0 ± 0

Score 1 least dominant and 5 most dominant

Correlation of color indicator towards pH, SSC and Texture

Figure 5 shows the pH values of the guava samples along with the color indicator response. The pH value of the guava varied from initially pH 3.94 at fresh ripe to pH 3.56 at overripe stage after 7 days (Fig. 5). Furthermore, the color indicator responded to the decrease in pH value, since the pH range of BPB/cellulose membrane color change is fit to the levels of the pH (3.5–3.9) in the guava samples.

This pH change (from 3.94 to 3.56) reflected the developing state of guava in this period, where mainly caused by acetic acid as the most volatile acid in guava. Since acetic acid was fermentative guava metabolites during this period. In this case, by enzymatic reaction (alcohol dehydrogenase), ethanol was oxidized into

acetaldehyde, which is then further oxidized into acetic acid [17]. In general, the acetic acid content of packaged guava at the ripe stage was higher than in normal guava (unpacked). Thus, apart from influences of packaging on fruit metabolism, it is obvious the packaging effect on the increasing of acetic acid content in the head space of guava packaging. Since acetic acid as the end product of fermentative metabolite of guava in the package head space seems to be accumulated. Generally, the correlation between the changes of color indicator towards the pH change (3.56–3.94) was given as linier correlation in Fig. 6, where the correlation coefficient (r) was 0.993. It shows that BPB membrane fits well with this pH range.

During guava fruit development in these experiments, the SSC of guava samples increased (Fig. 7). The SSC increased during guava development, particularly in the

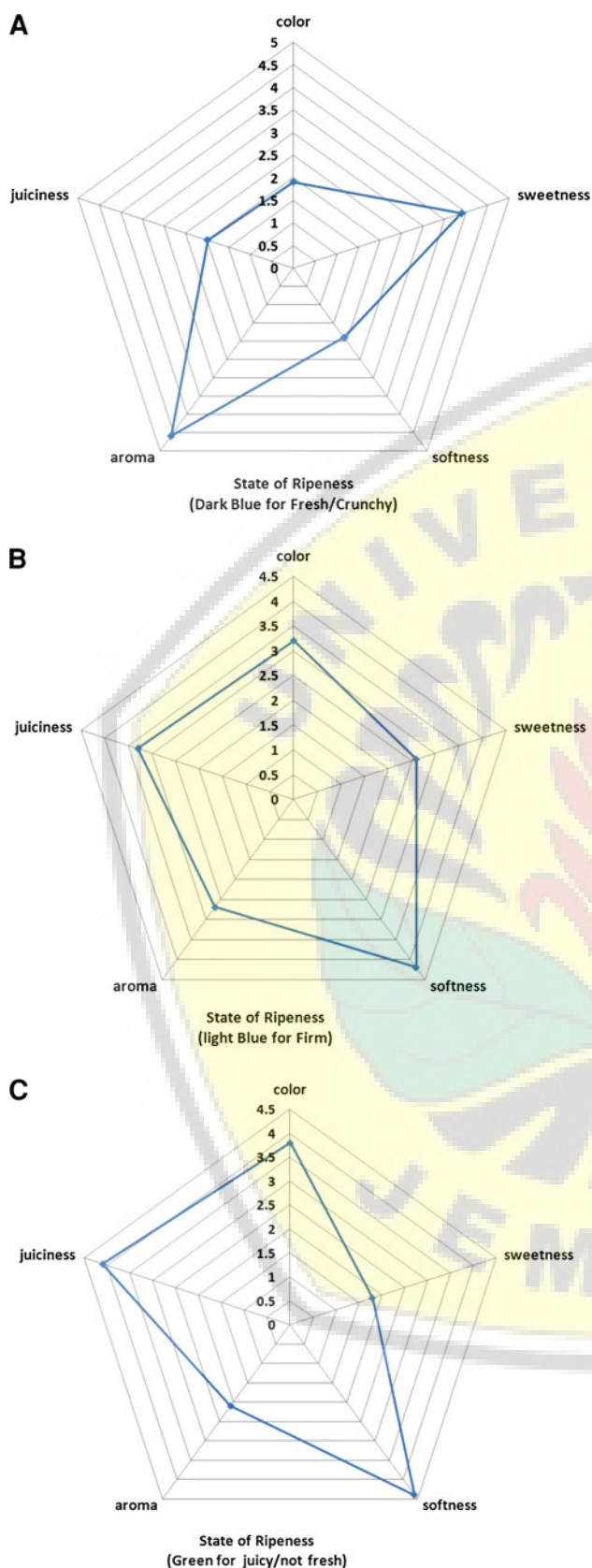


Fig. 9 Polar plot for guava ripening at the various state of ripeness according to the sensory evaluation (score from 1 to 5), **a** fresh (crunchy), **b** medium (firm) and **c** not fresh/overripe (juicy)

5 days of storage in ambient condition, is similar results to the others [16, 27, 28]. The remarkable increase in SSC may be attributed to increase in activity of the enzyme responsible for starch hydrolysis and for decline in the rate of sugar breakdown (e.g. glucose and fructose) by respiration [16]. In addition, since the guava in sealed condition, partial anaerobic respiration during storage might have also been resulted in the increased SSC. This is due to the fact that subtropical fruits including guava have been reported to be among the most sensitive to anaerobiosis damage [17, 29].

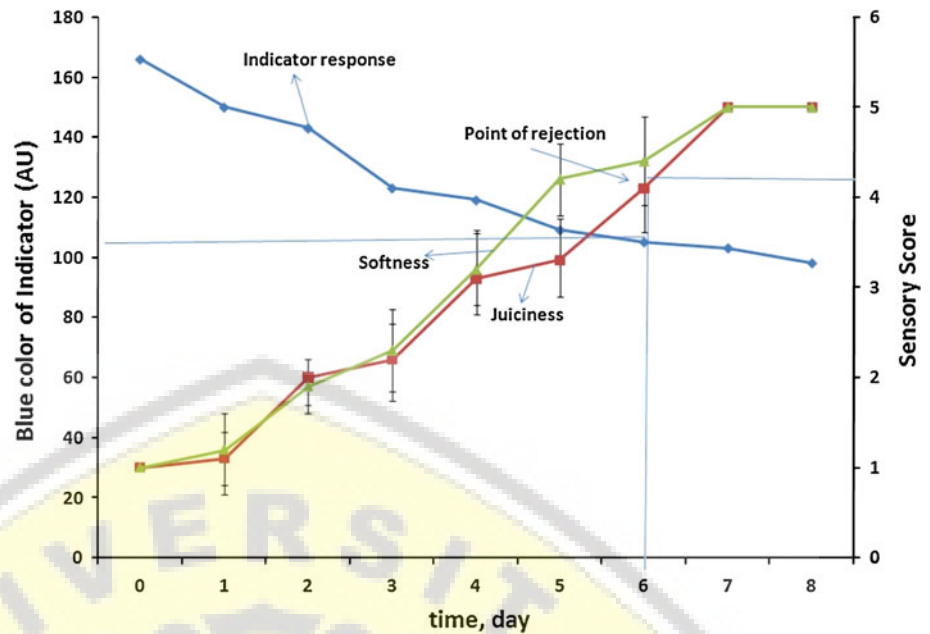
The texture of guava samples was conducted along with the color indicator response. Figure 8 is the average values of the texture readings for the measurements of texture softness of guava samples. There was an increase in the texture softness of guava fruit development, similar result was reported [16] in term of firmness of guava during ripening stage. Accordingly, it can be seen from both figures that the color indicator change showed similar trend with texture softness value. The ripening development, from ripe to overripe, along with increase in the texture softness of the guava samples, was in similar trend to the change of the color indicator, i.e. from blue to green. The increasing in texture softness or rapid softening of fruit has been proposed could be due to the increase in pectin solubilization and disruption xyloglucan-cellulose microfibril networks of guava fruit [30].

Correlation of color indicator and sensory evaluation

During ripening stage and development, a fruit including guava, passes through a series of over changes in color, texture and flavor, indicating that compositional change are occurring. Such chemical changes are needed to be accomplished for maximum eating quality of a fruit and for preferred ripeness. Ripe guavas are usually soft texture, sweet, non acidic, less astringent and highly flavored, so they are more acceptable for human food [16]. However, it is still difficult to determine its preferred sate of ripeness. Since, it is no exact criteria for certain state of ripeness. Therefore, the sensory evaluation is useful approach to evaluate the state of freshness, when the fruit was ripe.

Skin color changes in guava fruit were largely affected by storage condition [17]. Here, the guava had a drastic change in skin color as it changed from light green to greenish-yellow (Table 1). This is also reported [31], that the mature green guavas attained full yellow color in 11 days of storage at 12.5 and 15 °C. Furthermore, the loss of green color in guava is accelerated by increase in O₂ concentration in storage atmosphere [17]. As this also observed for normal guava, where the skin color change was slightly faster compared to packaged guava in room temperature. This slightly inhibition of loss of green color

Fig. 10 The sensory score (juiciness and softness) of guava samples and the indicator response



of packaged guava can be primarily due to the effect of packaging on ethylene sensitivity [32] for fruit and vegetable. However, packaged guava showed injury symptoms in the form of skin browning (Data not shown), which were further intensified during deterioration state. This injury could be due to the volatile induced by an aerobic state [17].

The sweetness of guava during ripening stage would be related to the hydrolysis of starch to sugar. This may be attributed to the increase in activity of enzymes responsible for starch hydrolysis and for decline in the rate of sugar breakdown by respiration [16]. Climacteric fruits may show considerable change in sugar content during fruit ripening, where starch and sucrose change into glucose during fruit ripening [33]. In the case of guavas, it showed that glucose, fructose and sucrose were the main sugars that responsible for sweetness [34]. In the case of guava ripening stage, where the sweetness increase, the juiciness will decrease, while in the case of overripe guava, where the sweetness decrease, the juiciness will increase and tend to mushy. This is due in the overripe guavas, the level of main sugar (glucose, fructose and sucrose) that responsible for sweetness was low [16] as this sugar was converted enzymatically to ethanol, oxidized into acetaldehyde, which is then further oxidized into acetic acid [17].

Aroma of guava is related to the accumulation of ethanol and acetaldehyde during ripening of fruits and contribute both to the removal of astringency and to the development of aroma volatile in fruit [29, 35]. Here, we speculate that acetaldehyde accumulation and its increase during ripening may be contributing to the aroma volatile compounds biosynthesis in guava. Furthermore, its role in the removal of astringency from guava, as a significant

reduction in the total phenols could be occurred during ripening stage [17]. While ethanol and acetaldehyde contribute significantly to inhibit ripening and flavor quality, but their biosynthesis in excess leads to off-flavor development in fruit [29] along with increasing of acetic acid concentrations as the end product of these guava fermentative metabolites.

The softness of guava was similar trend toward texture softness measurement by the instrument (texture meter), where an increase in the softness of packaged guava fruit stored in ambient condition was also observed [16]. Fruit development occurs along with the increase softness of the guava samples. The rapid softening of fruit has been proposed could be due to the increase in pectin solubilization and disruption xyloglucan-cellulose microfibril networks of guava fruit [30].

In general, the correlations between the changes of color indicator toward the results of classification of freshness criteria in the sensor evaluation are given as polar plot in Fig. 9, as also reported by Mota et al. [36] for stone fruit (e.g. kiwifruits). As estimated for typical ripeness stage of guava, the yellow color, softness of skin and juiciness increased, whereas sweetness and aroma content decreased as off-flavor development occurs in guava [29].

Figure 10 shows the output score of the juiciness and softness measurement corresponding to Tables 1 (The results of the sensory evaluation). It can be seen that the color indicator change shows similar response to sensory response (juiciness and softness score) where the point of rejection of sensory score was similar with the onset of detection of the color indicator response. Here, the juiciness and softness were selected as rejection indication, since in both cases, the guava tend to mushy, as the most

unacceptable to the panels or consumers. This is indicated by color change of the color indicator to green for overripe indication.

Conclusions

A simple BPB/cellulose membrane was used to develop on-package color indicator and the relationship between the indicator and guava freshness state was investigated. The test results show that the color indicator could be used for assessing guava freshness state and the correlation between the color change of the indicator and the freshness state of guava is in similar trend, and the deterioration of the guava samples could be detected clearly (when the color indicator change to green). The color indicator reacts toward pH change as the volatile organic acids (e.g. acetic acid) produced gradually in the package headspace during development of guava. Thus for the producers, the color indicator is mainly used to determine the deterioration period and the period of salability of guavas packaging. Whereas for the consumers, by viewing the color of the indicator, it could aid to choose guava at their preferred freshness state. In term of cost, this color indicator is really cheap, its cost was estimated to be around fifteen cent (USD), while in the mass production, it could be less. Furthermore, the color indicator may serve as an active shelf-life labeling device in conjunction with the “used-by-date” labeling, when attached to individual packaged product unit, or may be used to optimize distribution control and management of the stock rotation system, and reducing fruit waste.

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