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The use of colour indicator as a smart packaging system for evaluating mangoes Arummanis (*Mangifera indica* L. var. Arummanisa) freshness

A Dirpan[1], R Latief[1], A Syarifuddin[1], A N F Rahman[1], R P Putra[2] and S H Hidayat[1]

[1] Department of Agricultural Technology, Faculty of Agriculture, Hasanuddin University, Jalan Perintis Kemerdekaan KM 10, Makassar, 90245, Indonesia.
[2] Department of Agriculture Technology Education, Faculty of Engineering, Universitas Negeri Makassar, Jalan A. P. Pettarani, Makassar, 90222, Indonesia.

E-mail: *dirpan@unhas.ac.id*

Abstract. The high demand and public sensitivity of packaging products need the emergence of various packaging for fresh fruit and vegetables. One of the packaging that can be applied is smart packaging. The aimed of this study was to make smart packaging indicator that can be applied to mangoes. The benefits of this research were to facilitate the consumer to know the quality of the fruit and to promote in choosing the fruit according to freshness or desired maturity without damaging the packaging. The research was carried out in the following two stages: preparation of solution as bacterial growth medium *Acetobacter xylinum*, (incubated for 14 days) and making colour indicator and application to smart packaging. The results showed that long immersion of bacterial cellulose with bromophenol blue solution was 24 hours and temperature of ± 30 °C. The colour indicator that has been applied to the packaging changed from blue to green colour for over-ripe product, which can be visible to the naked eye. The colour change of the indicators reflects the pH of the mango packaging. It was also in similar trends to the change of several parameters (total acid, and soluble solids content (SSC)) that was typically used to characterize the freshness of mango. In general, this indicator can be used as smart packaging.

1. Introduction

There have been more demands from the consumers to have food products with high quality in developed countries. This led to the emergence of various innovations in the field of packaging technology that allows consumers to choose fresh fruit and vegetable products that have been packaged[1, 2]. One of the packagings that can be applied is smart packaging [3, 4]. Smart packaging is the package function switches on and off in response to changing external/internal conditions and can include communication with the customers or end users as to the status of the product. Therefore, intelligent packaging can be stated as a system that monitors the condition of the packaged food to provide information about the quality of transport and distribution [4, 5].

Some studies on constructing and applying smart packaging have been conducted. Kuswandi and Nurfawidi [6] have developed the label that can be used as a simple and practical indicator for freshness monitoring in packaged beef. In their study, the label responses accurately to the beef freshness, in term of its pH change due to the deterioration as shown by both colour change at room and chiller temperatures. Similarly with the other parameters, such as the total volatile basic amine (TVBN), total viable count (TVC) and sensory evaluation. These levels were reached at eight hour and seven days as the threshold of spoilage at room and chiller temperature respectively. Cavallo *et al.* [7] investigated a colourimetric sensor of milk spoilage which was built from adsorption of methylene blue (MB) into a modified polypropylene film (PP). The results showed that devices were suitable sensors to be used in the development of smart packaging. Kuswandi *et al.* [8] created a real-time monitoring of shrimp spoilage using on-package sticker sensor based on natural dye of curcumin. The result shows that the curcumin/bacterial cellulose membrane was successfully used as a sticker sensor for real-time monitoring of shrimp spoilage in ambient and chiller conditions. Hong and Park [3, 9] constructed colour indicators for kimchi (fermented vegetable product) packaging. These colour indicators were developed to monitor the quality of such commercial products during storage and distribution. Kuswandi *et al.* [10] also have created colour indicator based on bromophenol blue and tests have been conducted to assess the freshness of guava (*Psidium guajava L.*). It can be seen that the indicator can be used for real-time visual monitoring of freshness state of packaged guavas. From the above studies, it can be concluded that smart packaging can be applied to the food product including fruit.

The application of smart packaging can also be applied to mango fruit. This is because of the mango fruit including climacteric fruit group. Climacteric fruits do not need to be harvested when the ripe fruit is full in the tree because the fruit picked at the level of ages before ripening in the trees can be perfectly ripe after being stored. At the time of transportation with the distance, far enough climacteric fruit can be damaged. Due to the increase in temperature inside the crate, this comes from heat released by the fruit plus heat from the outside environment which can cause more rapid damage.

To the best of the author's knowledge, this is the first study to create smart packaging on mango arummanis, particularly in South Sulawesi, Indonesia. Therefore the purpose of this study were: (1) To create and study the indicator of freshness as an element of smart packaging (smart packaging) on mango arummanis fruit (2) To know the profile of colour change on freshness indicator of mango arummanis fruit.

2. Material and Method

This experiment was conducted in January - May 2017 in Microbiology and Food Biotechnology Laboratory and of Food Quality Analysis Laboratory, Food Science and Technology study Program, Department of Agricultural Technology, Hasanuddin University, Indonesia.

2.1. Preparation of Cellulose Membrane

As previously described [8, 10, 11], membrane sheets were prepared with some modification from bacterial cellulose (10 g), blended until homogeneous, then cast into the glass plate, pressed and left overnight (12 h). Finally, the white membrane sheet dried at 60 and ready to be used.

2.2. Immersion of Bacterial Cellulose

Bromophenol blue (BPB) was prepared by dissolving 10 ml BPB in 10 ml of ethanol (70%). After obtaining the cellulose membrane, the bacteria are then added with a 5% NaOH solution, for 60 min at 100 °C to remove the bacterial cell and substrate from the layer in the bacterial cellulose film. Then rinsed with tap water until reach neutral condition or pH 7.0. The bacterial cellulose membrane layer was stored in a glass plate, pressed and allowed to overnight (12 hours). A white membrane sheet was dried at 60 °C and ready for use [10].

2.3. Preparation of the Colour Indicator Labels

Colour indicator labels were produced by immersing the membrane sheet into 10 ml of BPB solution (1 mg/ml) at ambient condition (28 ± 2^0C) for overnight (12 h). Practically, the successful immobilization of BPB into bacterial cellulose membrane was shown by the dark blue colour of the membrane as the original membrane colour 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 to give the dark blue colour). The membrane was then dried with an electrical drier (Philips) and cut into the desired shape [10].

2.4. Smart Packaging Application on Mango
Mango weighing about 200 g was used for analysis, then placed on a styrofoam and covered with polyethene plastic film. Samples were stored in room condition with normal light exposure. pH analysis, total soluble solid content (SSC), total acid. The colour change index was performed every 48 hours.

2.5. Measurement of SSC, pH and Total Acid
The determination of soluble solids content (SSC) was performed by homogenized the mango (three replications) and the homogenate filtered through several layers of cheese cloth to obtain a clear juice. The SSC (%) was recorded with a refractometer (Model: PAL-1, Otago co, Ltd, Tokyo, Japan). The pH values were recorded by a pH meter.

Total acid was measured acid by a titration method with 10 ml suspension plus 2-3 drops of phenolphthalein indicator, then titrated with 0.1N NaOH solution until the colour turns to light colour and the colour does not change back for 30 seconds. At the end of the titration, the amount of NaOH used was calculated. Finally, total acid was calculated using this equation:

$$\% \text{ total acid} = \frac{V\ NaOH\ x\ N\ NaOH\ x\ FP\ x\ Grek}{1000\ x\ sample\ weight}$$

2.6. Measurement of the Colour Indicator Using Colour Analysis
The colour indicator was placed inside the package of the mango samples attached to the plastic wrap of the package using double transparent tape, where the indirect indicator contact with the atmosphere inside the package and covered with the label for viewing the colour of the indicator along with the reference colour for the state of freshness. Then the samples were stored in ambient condition. Besides, the colour indicator can be viewed by the naked eye in term of the colour change during mango deterioration state. For quantification of colour measurement of the indicator, a simple method was used using a digital camera [10]. A microscope digital (model: digital microscope 5-500 x) was used to record the colour of the indicator. The graphics software CorelDrawx6 was then used to analyze the colour of the indicator. The term ''measure'' means that the digital camera is used to obtain the colour values of the pixels on the colour indicator membrane. The term ''analyze'' means that CorelDraw is used to measure those colour values to obtain colour distribution, averages, and so on. Here, we used the 'Eyedropper Tool' for the colour value of a selected area in the membrane image of the colour indicator. The blue value was used as the colour value for all membrane colour measurements, since the membrane colour change from blue to green. By using this method, it is more suitable and simpler as compared to the visual inspection by the naked eye as the main purpose for this indicator rather than using reflectance measurement [10, 12]. The principles of colour measurement can be found elsewhere [10, 13].

2.7. Statistical Analysis
SSC, pH and total acid values were analyzed by one-way analysis of variance (ANOVA) while the significance of differences among means was determined using Tukey's test with a level of significance of $P < 0.05$. SPSS software for Windows version 16.0 (SPSS Inc., IL, USA) and Microsoft Excel 2010 was used to analyze the data.

3. Results and discussion

3.1. pH
The aim of the pH determination was to determine the acidity of mango fruit and to study the effect of pH on the freshness indicator of mango fruit.

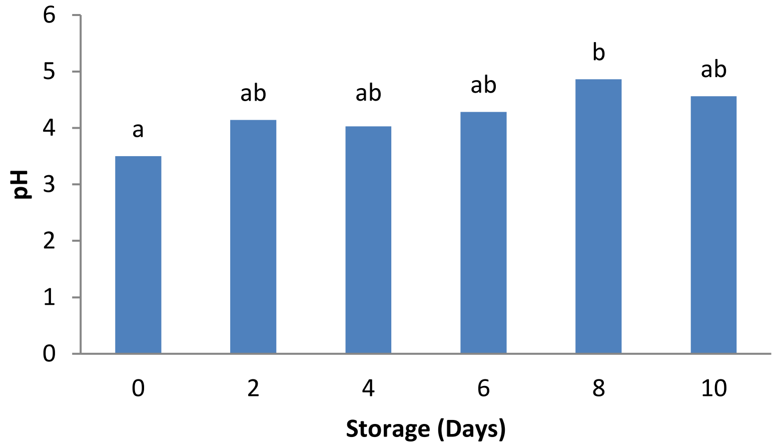

Figure 1. Changes in pH for Mango fruit at different storage

Figure 1 explains the typical diurnal changes of pH from 0 to 10 days storage. It can be seen that there is an increase in pH every day during storage. The results of storage on day 0 showed the pH value of mango around 3.5 while on day 8 showed pH value of 4.7. The occurrence of pH increase in mango fruit is caused by the influence of long-time storage, enzymatic reactions, and microbiological changes. Also, Arumanis Mango stored at room temperature showed increase in pH value or decrease in acidity. Furthermore, higher acid content is present in maturation fruits than in ripe and decaying fruit. This is by the statement Muchtadi *et al*. [14] which states that maturation fruits contain lots of organic acids and will decrease during the fruit ripening process.

3.2. Total Acid

Total acid is the overall amount of acid contained in a material. Total acid associated with pH but different principles. The pH level measures the total strength of the acid, while the total acid measures the amount of acid.

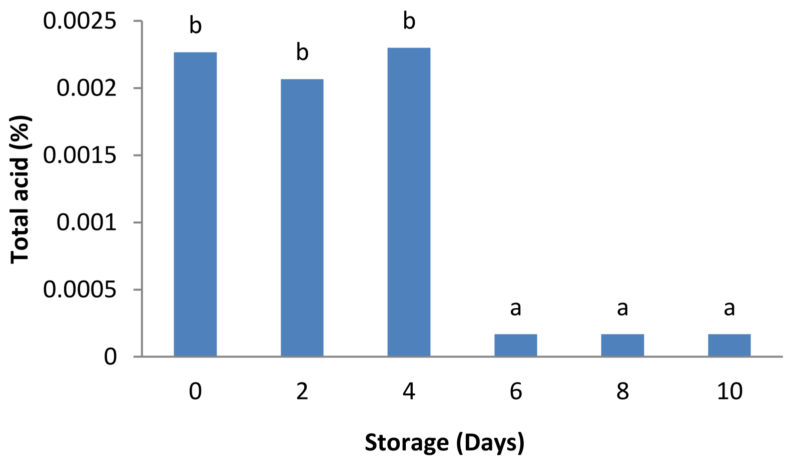

Figure 2. Changes in total acid for Mango fruit at different storage

Figure 2 shows the total acid on mango fruit during storage. The difference of total acid values in ten days storage was detected significantly by Tukey's test at 5% level ($P < 0.05$). The highest total acid occurred at 0 days, the average value of total acid was about 1.2%. This value was decreased significantly until ten-day storage. Differences in total acid could be due to the use of organic acids in the mango fruit by process of respiration that requires energy. The energy required is derived from overhauling the nutrients contained in the food. Also, a decrease in the total acid value of the fruit is due to the absence of an enzymatic reaction (alcohol dehydrogenase), the ethanol is oxidized to acetaldehyde, then oxidized to acetic acid during ripening. Thus, regardless of the influence of fruit metabolism, the fruit maturity level also affects the acid level. This is in line with the statement of Muchtadi *et al.* [14] which states that maturation fruits contain lots of organic acids and will decrease during the fruit ripening process.

3.3. Soluble Solids Content (SSC)
The figure 3 showed that mango fruit has total soluble solid at 0 days storage is 5.4%, and 13.0% at ten days storage. Based on these data it is known that during the storage, the total soluble solid in the mango fruit increased slightly. This is due to the change of starch in the mango into sugar so that the sugar content in the fruit increases during storage. This is consistent with the statement Pandarinathan *et al.* [15] that increased sugar content in fruit during the ripening process will increase the total value of the SSC, the formed sugars will be used as energy for respiration. Also, the packed mango fruit will undergo an anaerobic respiration process that also will increase the SSC.

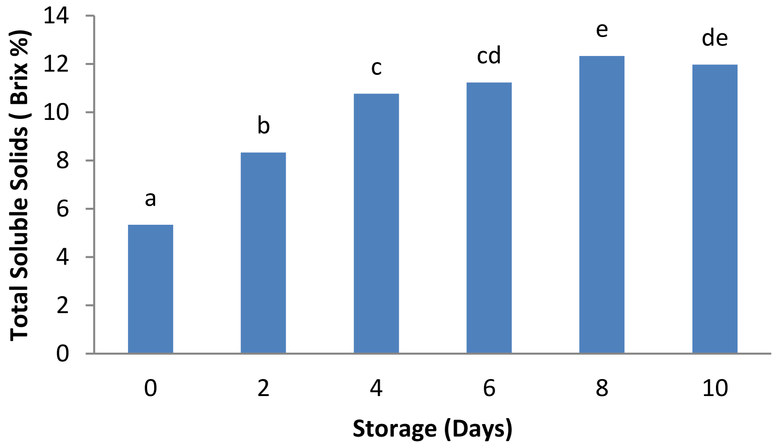

Figure 3. Changes in total soluble solid for Mango fruit at different storage

3.4. Colour Changes Index
Figure 4 showed the rate of colour changes of the indicator. It can be seen that the colour indicator response decrease steadily (as the indicator colour change to green) within ten days of the experiment was observed. Here, the colour indicator gradually changed colour from blue to green after six days. The colour indicator response as the colour change was similar with deterioration state of the climacteric nature of mango that was well reported in the literature [10]. The onset of overripe was detected after six days of storage. This indicated that the packaged mango released volatile acids (e.g. acetic acid) at a relatively slow rate in comparison with normal mango since its freshness lasted longer within six days. On the other hand, the packaging influence on other acid compounds in the mango, such as ascorbic acid, was found positive and effective in preventing the losses of this acid in fruit during ripening stage [10, 16, 17].

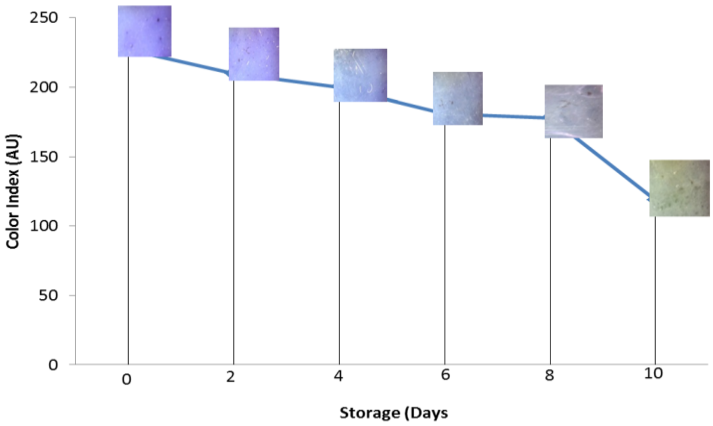

Figure 4. The rate of colour changes of the indicator response towards mango freshness state

4. Conclusion

1. Freshness indicator of mango fruit can be produced by utilizing bacterial cellulose from *Acetobakter xilinum* which then soaked with bromophenol blue solution.
2. Profile of colour change on the freshness indicator of mango arummanis showed the change of colour from dark blue indicating fresh fruit, light blue is firm and green colour indicates the fruit has been rotten

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