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Original Research Article

Immobilisation of Anthocyanin in Starch-Based Film as a Potential Colorimetric Oxygen Indicator in Modified Atmosphere Condition

Nurul Husna Che Hamzah[1], Nozieana Khairuddin[1,2]*, Ida Idayu Muhamad[3]

[1]Department of Science and Technology, Faculty of Humanities, Management, and Science, Universiti Putra Malaysia, Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia, husna.chehamzah@gmail.com

[2]Institut Ekosains Borneo, Universiti Putra Malaysia Bintulu Sarawak Campus, Bintulu 97008, Malaysia, nozieana@upm.edu.my

[3]Department of Bioprocess and Polymer Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia, idaidayu@utm.my

**Corresponding author: Nozieana Khairuddin, Department of Science and Technology, Faculty of Humanities, Management and Science, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia.; nozieana@upm.edu.my

Abstract: Modern food packaging implies modified atmosphere packaging (MAP) to prolong the shelf life of food. Certain foods need oxygen such as meat (myoglobin) red colour. Recently, colourimetric oxygen indicator has been beneficial to be applied in food packaging because it can be used to monitor the oxidation in the headspace of MA packaging). Anthocyanin pigments are sensitive to gases. In this study, different concentration of red cabbage anthocyanin (RCA) was incorporated in sago starch formulations to produce an oxygen indicator film. A modified atmosphere environment was set up by using a desiccator connected to a vacuum pump to remove the oxygen gas until the required level (10%). The oxygen level was monitored using an oxygen meter. It was observed that the films were able to be used for oxidation process detection since the anthocyanin changed its original purple colour to brown once it was exposed to the oxygen, whereas the original colour of the films can be preserved in a modified oxygen atmosphere which has an oxygen content of less than 10%. The colour properties of the indicator films were recorded using the CIELAB scale (L*, a*, b*) which signifies lightness, redness-greenness, and yellowness-blueness, respectively. A film with 12% w/v RCA showed the highest colour lightness transition in a modified oxygen atmosphere (1.63%) whereas the 8% w/v RCA film showed the highest lightness transition in normal oxygen conditions (2.25%). The prepared oxygen indicator film was able to provide visual support to judge whether there is an oxidation process that has occurred in the food packaging closed system.

Keywords: anthocyanin; modified atmosphere packaging (MAP); oxygen indicator; intelligent packaging; *Brassica oleracea*

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1. Introduction

Active and smart packaging is a novel food packaging technology that is rapidly increasing in this century. Active packaging is packaging material that can protect the quality or safety of the products such as ethylene absorber or inhibitor, modified atmosphere packaging (MAP), and oxygen absorber. Meanwhile, smart packaging has a function to monitor or track the change in the external or internal environment of packages and communicate the condition of products to customers such as ripeness indicator, ready-to-serve indicator, food spoilage indicator, time temperature indicator, and oxygen indicator (Suwannawatanamatee *et al.*, 2018). An oxygen indicator is a tool for checking or monitoring the oxygen-sensitive food that was packed using vacuum packaging (VP), and modified atmosphere packaging (MAP).

An oxygen indicator is categorised as a gas sensor that is used for detecting the presence of a gaseous analyte in the package (Biji *et al.*, 2015). It also acts as an integrity indicator because it can sense the leak in the packaging during the production and distribution chain (Realini & Marcos, 2014). Oxygen promotes aerobic microorganism proliferation, the formation of off-flavours, odours, colour changes, nutritional losses, and general shelf-life stability of foods for muscles (Realini & Marcos, 2014) To slow down the rate of these food spoiling events, it is crucial to regulate the oxygen levels in food packaging. Although MAP or vacuum packaging can be used to store goods that need to be oxygen-free, the oxygen is not entirely removed. Thus, oxygen-level monitoring is essential to be incorporated into the food packaging to ensure the quality of the food. Three types of oxygen detectors have been integrated into packaging film. The first type is based on luminescent probe molecules embedded in polymer films that are permeable to oxygen. Colourimetric redox based indicators represent another option. Another type is phosphorescent sensors which rely on the principle of quenching luminescence intensity and a lifetime of a dye embedded in a polymeric matrix (Zhang *et al.*, 2022).

Sago starch is an uncommon and cheap source of starch that has many benefits that are preferable to be used as packaging film (Arezoo *et al.*, 2020). Sago starch has unique characteristics and some of its physicochemical properties are quite similar to common starches such as cassava and potato (Abral *et al.*, 2019; Fazilah *et al.*, 2011; Mohammadi Nafchi *et al.*, 2011). The starch can be incorporated with many kinds of natural materials to function as intelligent packaging. Plants such as red cabbage (*Brassica oleracea)* are the most common source of anthocyanin extraction, the natural colourant that exists in plants. It is very sensitive to pH changes and easily oxidised when exposed to oxygen due to chemical degradation during storage (Chung *et al.*, 2016). The red cabbage anthocyanins exhibited the highest heat and pH stability compared to other anthocyanin sources (Ekici *et al.*, 2014). Hence, this work aimed to investigate the developed sago starch and red cabbage (*Brassica oleracea)* as an oxygen-level monitoring film.

2. Materials and Methods

2.1. Materials

The red cabbage (*Brassica oleracea*) powder was obtained from a local distributor (Hana Natural, Malaysia). The starch was bought from the local store in Bintulu, Malaysia. The glycerin was purchased from Merck Malaysia. Distilled water was used throughout this work.

2.2. Film Preparation

A solvent-casting method was used in this study. Firstly, the red cabbage extract solution was prepared using the following three different weights (8, 10, 12, 14, and 16 g). The red cabbage extract (RCE) was stirred and dissolved in 100 mL of distilled water until the solution became homogeneous. Then, the solution was heated until mild boiling before adding 5 g of local sago starch and 3 mL of glycerin (HmbG Chemicals, Germany) into the solution. The solution was stirred well until the temperature reached about 75°C or until the solution became gelatinised and viscous. Lastly, the films were cast into a square plate (20 × 20 cm). The casting plate was placed for 3 to 4 h in an oven (Memmert, Germany) set at 60°C. The control film was prepared with no RCE being added to the film solution. The films produced were peeled off and stored in a dry desiccator before further testing and analysis. Table 1 summarises the ratio of starch and RCE in the formulation.

Table 1. Amount of RCE anthocyanin in each film

Films	Ratio starch to RCE	Starch (g)	Glycerin (g)	RCE (g)
Control	-	5	3	0
8% RCA	1: 1.6	5	3	8
10% RCA	1: 2.0	5	3	10
12% RCA	1: 2.4	5	3	12
14% RCA	1: 2.8	5	3	14
16% RCA	1: 3.2	5	3	16

2.3. Modified Oxygen Atmosphere Condition System

A desiccator was used as a closed system for monitoring the colour changes of films in a modified oxygen atmosphere. A hose was used to connect the desiccator with a vacuum suction pump (Ulvac Kiko. Inc., Japan) to control the oxygen concentration inside the desiccator. When the pump starts to suck the oxygen out from the desiccator, an oxygen detector AR810 (Intell Instruments Smart Pro, China) was placed inside to read the oxygen concentration until the desired amount (10%). After that, the suction process was stopped by turning around the opening or hole on top of the desiccator to block the oxygen from going out from the desiccator. Another film was also placed in normal surroundings which has oxygen content (21%). Figure 1 shows the setup for this experiment. The temperature in the normal surroundings and inside the desiccator was set to room temperature (23°C).

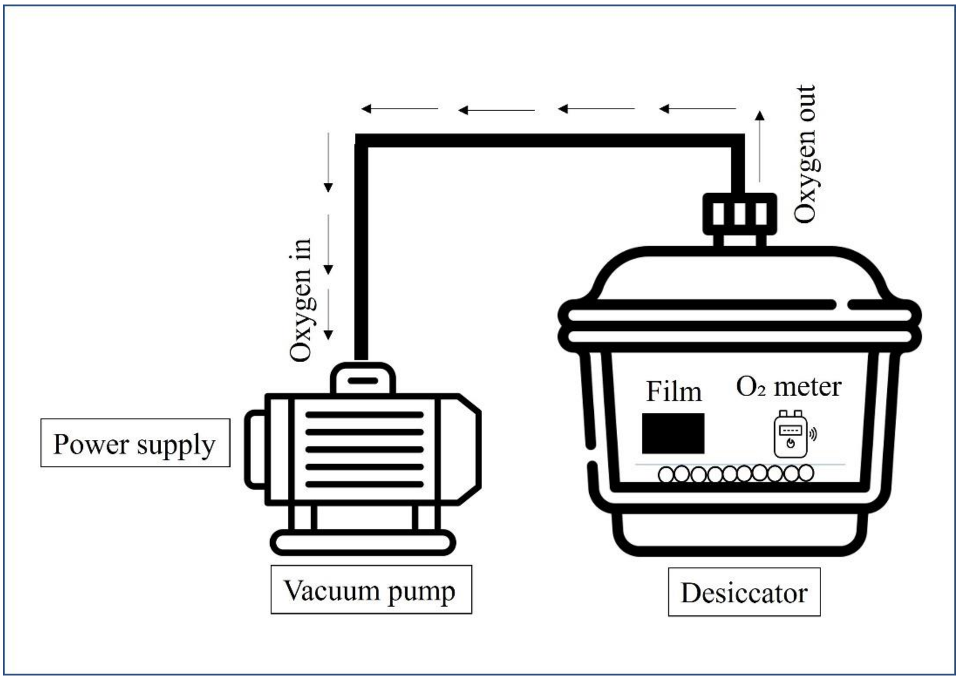

Figure 1. Experimental setup for modified oxygen atmosphere system

2.4. *Colour Changes of Films in Different Oxygen Levels*

The colour changes of smart films in both surroundings were measured and compared using a Color Reader CR10 (Konica Minolta, Tokyo, Japan) and measured using CIELAB colour space. The colour changes were measured every two days. The films were cut 3 × 3 cm and placed on the wall of the desiccators inside. The films were glued to the walls so that the colour changes could be observed and measured correctly using the portable Color Reader. The colour properties of oxidised films were recorded and analysed according to the specific CIELAB parameters such as lightness: L*, greenness to redness: a*, and blueness to yellowness: b*.

3. Results and Discussions

3.1. Visual Observation on the Colour Changes of Films

Figure 2 shows the observation of the colour changes of films before and after oxidation. After 11 days of exposure to normal oxygen, the colour of the films changed to brownish which indicated that the oxidation process had occurred. The fading of the original colours was due to the chemical degradation of anthocyanin after exposure to many factors including pH, light, temperature, oxygen, enzymes, and ingredient interactions (Bordenave *et al*., 2014). Hence, the film used in the study can possibly act as an oxygen level indicator in advanced food packaging. As the anthocyanin content increased in the film, the lightness properties of the films decreased. Similarly, the opacity or transparency of the films also decreased significantly as shown in Figure 2.

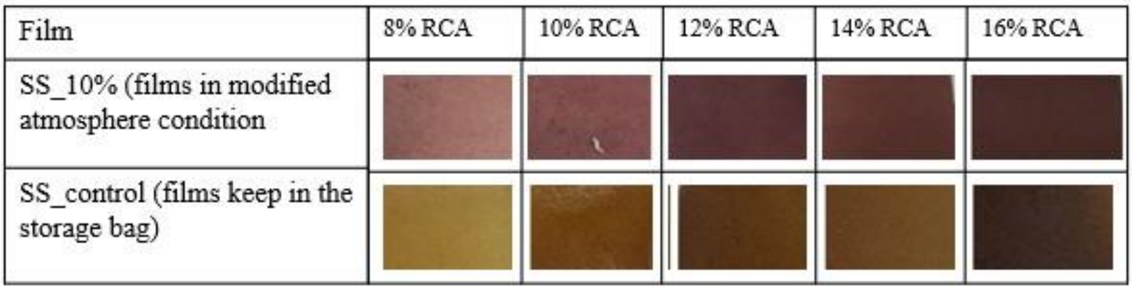

Figure 2. The film's colour changes in the modified atmosphere surrounding (original film) and after oxidation in normal oxygen conditions (oxidised film) after 11 days

3.2 Oxidation Based on the Colour Properties of Films

The existence of oxygen can lead to accelerated degradation of anthocyanins which can affect the colour properties of film (Oluwasina *et al*., 2019). The films that were exposed to normal oxygen surroundings showed a different trend of lightness properties compared to the films in the controlled environment (Figure 3). The films stored in modified oxygen conditions did not change the lightness, L* values for 11 days. However, the films started to change their colours to brownish when they became oxidised in an open area system. Polyphenol-oxidase and peroxidase are responsible for the enzymatic browning in plant and vegetable tissues (Marszałek *et al*., 2017). Therefore, the higher content of anthocyanin which was at 16% RCA film showed the most stability because the lightness values slightly increased during the time. However, the most preferable concentration of anthocyanin as an oxygen level indicator was within 8% to 12% w/v RCA since it showed the highest lightness values transition in both modified and normal oxygen conditions. Consumers will be more alert when the indicator is more sensitive to oxygen exposure.

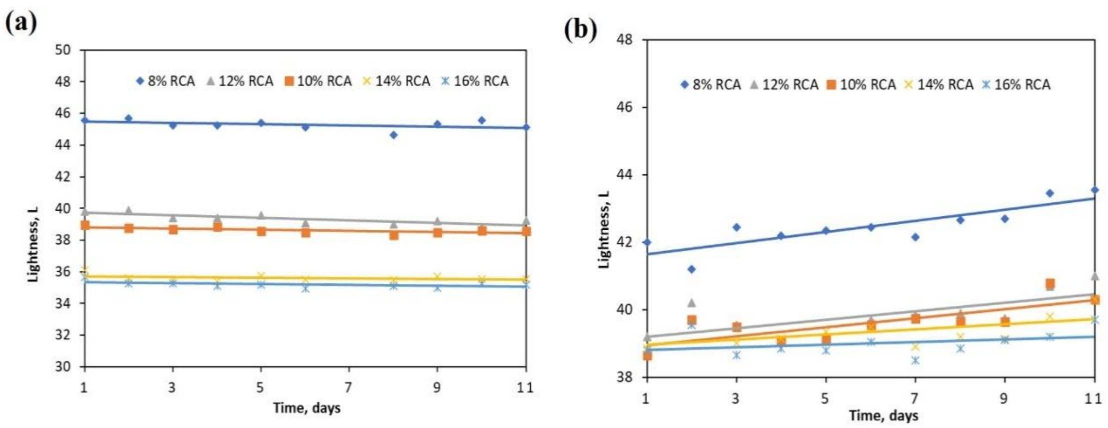

Figure 3. Lightness of films in (a) modified oxygen and (b) normal condition

Figure 4 displays the graph for greenness to redness properties of films, *a. In a modified oxygen condition, the redness to greenness values, a* was within the range of 3 (lowest redness) to 9 (highest redness). As can be seen in the Figure 4, the shifting of this colour attribute happened in normal oxygen conditions after a storage time indicating the colour change of films. It was observed that 16% RCA film shows a significant decrease of a* which shows the opposite trend with other film samples. This is probably due to the transition into the dark brownish colour of the films instead of the lighter brownish of other film samples. The increasing trend of blueness to yellowness, *b** indicates the increasing yellowness index as shown in Figure 4 (b). The highest content of anthocyanin (16% RCA film) showed the lowest *b** because it has a deeper yellow colour compared to other film samples (Zhai *et al*., 2018).

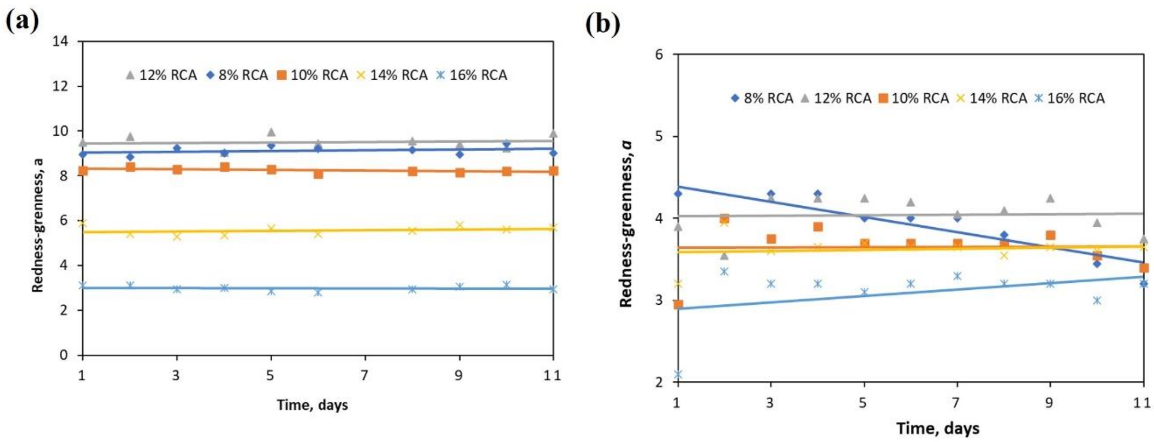

Figure 4. Greenness to redness properties of films, a* in (a) modified oxygen and (b) normal condition

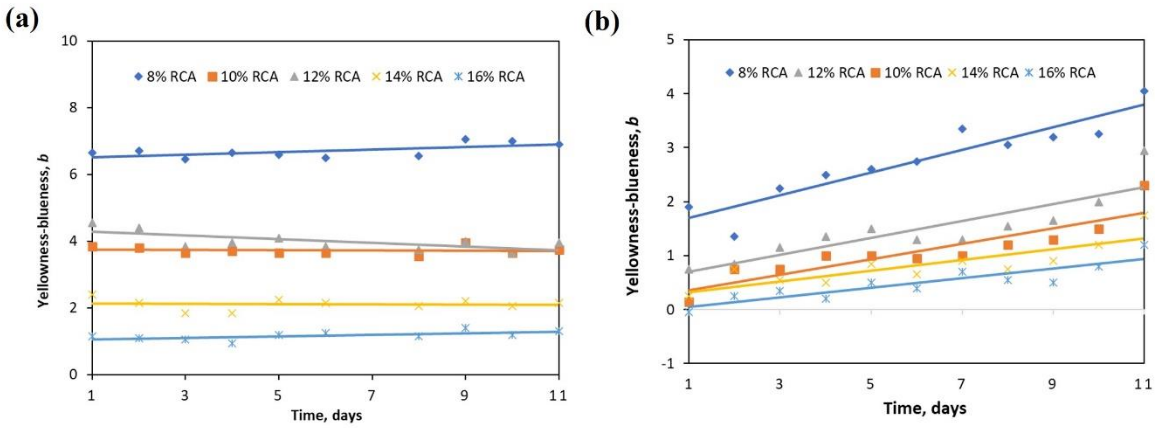

Figure 5. Blueness to yellowness properties of films, *b in (a) modified oxygen and (b) normal condition

4. Conclusion

The present study has discovered the potential of anthocyanin-starch-based films as oxygen level indicators since the films have oxidation properties when exposed to normal oxygen surroundings. However, in modified oxygen surroundings, the films can keep their original colours. Thus, it is applicable for oxygen level monitoring that will prevent the food from deteriorating faster. It can be concluded that a higher concentration of anthocyanin incorporated in the starch formulation has caused slower oxidation which simultaneously caused a small transition of colours. Thus, a lower anthocyanin concentration could be applied as an oxygen level indicator in the food packaging industry due to the fast transition of colours when exposed to normal oxygen. Further study is required to determine the barrier and mechanical properties of this intelligent film based on *Brassica oleracea* and sago starch to function well as an oxygen level indicator.

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