Shelf Life and Secondary Metabolite Content of Sweet Potato (*Ipomoea batatas L.*) Lam. Coated with Chitosan Coating at Low Temperature Storage

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Abstract

Sweet potato (*Ipomoea batatas* (L.) Lam.) has diverse varieties with different secondary metabolite content. Post-harvest treatment with low-temperature storage and chitosan coating is expected to expand the shelf life of sweet potatoes. The combination of these treatments will affect the secondary metabolite content of diverse sweet potato varieties. Therefore, this study aims to observe the secondary metabolite content and shelf life extension of 3 sweet potato varieties after coating with chitosan and low-temperature storage. A completely randomized design (CRD) was used with a three-factors experiment. The first factor was chitosan concentration at 0, 10, 15, and 20 g/L, the second was storage room temperature at 25, 15, and 5 °C, while the third was the color of sweet potato varieties namely white, purple, and orange from Tembakur and Mendut varieties. Meanwhile, the control group was tubers without chitosan coating at a storage temperature of 25 °C. Each treatment had five replications and the parameters assessed were changes in wet weight, hardness, respiration, the total chlorophyll level, carotenoid, vitamin C, reducing sugar, and the level of flavonoid. Data were analyzed with Analysis of Variance and then continued with Duncan's Multiple Range Test at a significance level of 5%. The result showed that low-temperature storage combined with chitosan coating affected the shelf life of sweet potatoes. Overall, the best storage temperature was 15 °C, indicated by the highest residual secondary metabolite and the most extended shelf life. The 5°C treatment decreased oxygen consumption during storage, as indicated by a low respiration rate. However, this storage temperature caused a chilling injury and culminated in the shorter shelf life of all examined sweet potatoes. The best coating was achieved by chitosan 15 g/L, indicated by the capability to coat sweet potato surface and maintain the high content of all targeted chemical components. The results also revealed that 20 g/L chitosan concentration is not practical for coating due to its in elasticity and the potential to create a crack in the coating layer.

Keywords: Chitosan; sweet potato (*Ipomoea batatas* (L.) Lam.); low temperature; flavonoids

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INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is an alternative local food ingredient to reduce the reliance on rice and wheat. According to Barbara et al. (2018), it contains nutrients such as carbohydrates, minerals, and vitamins. Furthermore, yellow and purple sweet potato contains beta carotene and anthocyanins, which have the potential as antioxidants. Many varieties are characterized by different colors on the tubers, such as white, orange, and purple. The differences affect the physiological and biochemical characteristics during post-harvest storage.

After harvesting, the tubers conduct respiration to obtain energy (Ostergaard, 2010), and ripening until post-harvest involves the activity of enzymes. This biological compound controls respiration, biosynthesis, and degradation of hormones, including ethylene (Pua & Davey, 2010). Environmental stress, such as temperature, can affect the reaction rate and the resulting intermediate compounds, such as chlorophyll, vitamins, and flavonoids. Secondary metabolites are formed in response to environmental stress (Oladoye et al., 2016).

Coating techniques using edible polymers are often applied to the post-harvest handling of vegetables and fruits, such as chitosan coating. The coating on the tubers can provide mechanical strength to the peel and inhibit the entry of gases from the air into the peel or the exit of gases from the cells. This will reduce tuber water loss (Chailoo & Asghari, 2011), and chitosan is a natural material safe for consumption (Djioua et al., 2010).

Sweet potato tubers are sensitive to low temperatures (Choundary et al., 2010), a phenomenon known as chilling injury (CI). This sensitivity limits the use of low-temperature storage techniques that are effective in the post-harvest handling of tubers. Since each type of tuber has a different sensitivity to low temperature, it is necessary to study the effects of low temperature on physiological and biochemical changes, especially the secondary metabolites of sweet potato tubers in different varieties. Secondary metabolites are compounds released by cells in response to environmental stress. Therefore, studying which varieties are most susceptible to chilling injury is necessary. This research uses three varieties based on differences in the color of the tuber flesh, namely white, orange, and purple. The tubers were obtained from superior regional products and sweet potato cultivation centers in Kramen Hamlet, Matesih Sub-district, Karanganyar Regency, Indonesia. Most of the varieties planted are Tembakur and Mendut, and the three tubers have different secondary metabolite content. Orange and purple tubers have high carotene and antioxidants. Studying changes in the secondary metabolite content during treatment with chitosan coating and low temperature is necessary. Therefore, this research aims to determine the extension of shelf life and secondary metabolite content in three sweet potato varieties after being treated with chitosan coating and low temperature.

MATERIALS AND METHODS

Material

The materials used in this research are three color varieties of sweet potato, chitosan (E Merck deacetylation degree 95%), distilled water, acetic acid 1 ml/L, Whatman paper 42, sucrose, ethanol, aquabides, rocella salt, acetone, 0.1 N NaOH solution, ascorbic acid, $AlCl_3$, dinitrosalicylic acid, and quercetin (E Merck).

Tool

The tools are Mettler Toledo brand analytical balance, Thermo Scientific refrigerator, Lutron DO 5510 oxygen meter, penetrometer (Takemuratipe RHM-1), oven, porcelain cup, and mortar, spectrophotometer (Perkin Elmer's Lamda 25 series).

Research Design

This research uses a factorial completely randomized design (CRD) with three factors, namely chitosan concentration (K), shelf temperature (S), and sweet potato varieties based on color (P=White, U=Purple, O=Orange). Meanwhile, the chitosan concentration is at four levels, namely 0, 10, 15, and 20 g/L, while shelf temperature includes 5, 15, and 25°C. Sweet potatoes are selected from three varieties based on the white, orange, and purple colors from Tembakur and Mendut. The total treatment includes 36 combinations, where each is made with five replicates. The parameters are changes in fresh weight, total chlorophyll content, carotenoids, reducing sugars, hardness, thickness, respiration, vitamin C, shelf life, and flavonoid level. The data are analyzed by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at a significance level of 5%.

Procedure

In the first stage, white, orange, and purple sweet potatoes colors from Tobacco and Mendut varieties are prepared. The sweet potatoes are harvested from the same land at the harvesting age in Kramen - Pablengan Matesih hamlet. It is chosen from the same age and harvest region has the same weight range, and is disease-free. Subsequently, the sweet potatoes are washed under running water before drying.

Chitosan coating is made with a concentration of 10, 15, and 20 g/L. The 10 g/L is prepared by dissolving 10 g chitosan in 1 L of 1% acetic acid, stirring with a magnetic stirrer at 40 °C for 60 minutes until

the compound is dissolved. For 15 and 20 g/L, the chitosan was dissolved in 1 L of 1% acetic acid and can be removed with a vacuum after forming bubbles. Sweet potatoes are soaked in a tub containing chitosan solution with concentrations 0 (control), 10, 15, and 20 g/L for 10 minutes. It is shelved at room and refrigerator temperatures. Furthermore, the parameters are observed daily until the sweet potatoes are rotten or unfit for consumption, and the wet weight is weighed daily (Kurniawan et al., 2013).

Vitamin C analysis is conducted by weighing 5 g of sweet potatoes crushed with a mortar until smooth. Meanwhile, 10 mL of aquabides is added, homogenized, and filtered with Whatman 42 filter paper. About 4 mL of the filtrate is taken and analyzed with a spectrophotometer at a wavelength of 264 nm (Monalisa et al., 2013).

The respiration rate is measured by placing the samples weighing 0.5 kg (510.20 cm^3 volume) in a container of 2,400 cm^3 (15 x 16 x 10 cm). This treatment results in a free space of 1,889.8 cm^3, and the Lutron 5510 oxygen meter probe is inserted into the housing through the top lid, equipped with a valve. The sealed jars are put in the cooler according to the treatment, and the container is placed in the refrigerator at various temperature variations. Observation time is recorded, and oxygen concentrations are measured daily until day 25.

Chlorophyll and carotenoid levels are determined by crushing 1 g of flesh and adding 10 mL of 80% acetone. Furthermore, it is filtered with Whatman 42 filter paper, and the absorbance of the filtrate is measured at a wavelength of 480, 645, and 663 nm using a spectrophotometer.

The reducing sugar content is carried out using the DNS method, and the stock solution is prepared by dissolving 10 mg of anhydrous glucose in 10 ml of distilled water at a concentration of 0.2-1 mg/ml. The 10 g sample is mashed and dissolved in 100 ml before adding 1 ml in a test tube. Subsequently, 2 ml of 1% w/v DNS reagent is added, vortexed, boiled for 5 minutes, and cooled with water. After 1 ml of rocelle salt is added, a spectrophotometer will measure the absorbance at a wavelength of 540 nm.

The thickness of the chitosan coating is obtained with a micrometer by making cross-sections on the peel coated with the compound at various concentrations. Measurements and documentation are performed with digital microscope cameras.

Hardness is measured by placing the sample under a penetrometer needle for 5 seconds, and the load is removed before reading the pointer scale. This test is repeated at five points, and the average is calculated (Mudyantini et al., 2017).

Flavonoid levels are measured by weighing 15 mg of the extract. This extract is dissolved in 10 ml of ethanol and obtains a concentration of 1500 ppm. Furthermore, 1 ml of the extract is pipetted and added with 1 ml of

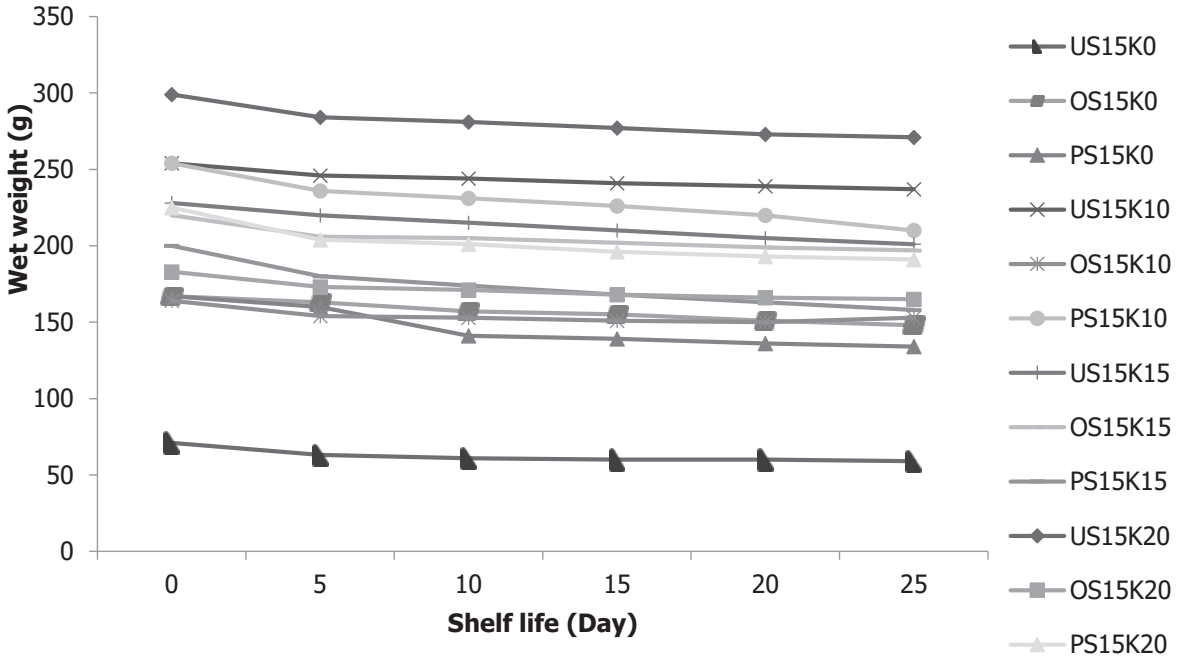

Figure 1. Wet weight of three varieties of sweet potato aged 25 days of shelf life at various concentrations of chitosan coating with a storage temperature of 15 °C. Note: U = purple, P = white, O = orange, S = temperature, K = chitosan

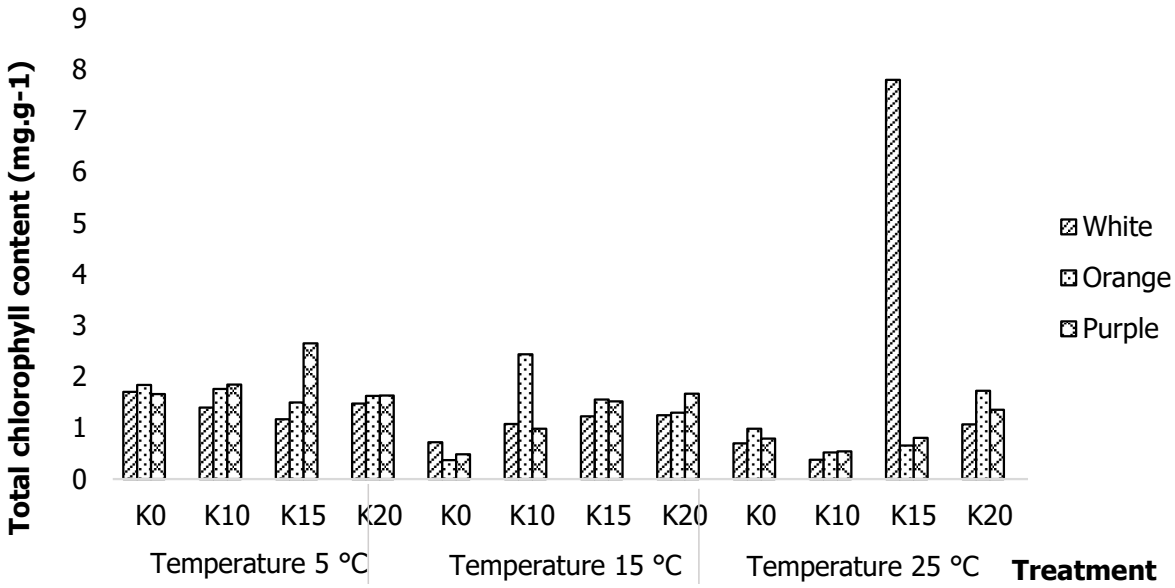

Figure 2. Total chlorophyll of three varieties of sweet potato aged 25 days shelf life at various concentrations of chitosan coating with variations in storage temperature

2% AlCl3 solution and 120 mM potassium acetate. It is incubated for one hour at room temperature, and the absorbance is measured at a wavelength of 435 nm.

Data Analysis

The measured parameters were analyzed with Analysis of Variance and then continued with Duncan's Multiple Range Test ($p < 0.05$) at a significance level of 5% using SPSS 20.0 version software to test the significance of difference among all treatments.

RESULTS AND DISCUSSION

Wet Weight of Sweet Potatoes

Figure 1 shows the results of wet weight measurements of sweet potato tubers throughout a 25-day shelf life at the optimal storage temperature of 15 °C.

At the beginning of low-temperature storage, the wet weight rate decreased, and the decline became more stable with the length of the storage period. This supported Huchin et al. (2013), where this change was an adjustment from room to low temperature. All treatments that were not coated with chitosan and stored at low-temperature C experienced a decrease in wet weight. Furthermore, the process of respiration causes loss of water in tubers. The storage at a low temperature of the mechanism of water loss occurs because the content in the tubers will be attracted to the surrounding air, which is cold and dry due to the low humidity of the cooling space.

Total Chlorophyll

Figure 2 shows the total chlorophyll content of three sweet potato types with a 25-day shelf life at varying chitosan coating amounts and temperatures.

Figure 2 shows that the total chlorophyll content in storage at 5 °C is higher than in other temperature treatments. Storage temperature is the main factor affecting chlorophyll degradation. A value below 15 °C causes slow chlorophyll degradation during storage (Shehata, 2012). The mechanism of reducing the degradation due to chitosan coating inhibits the entry of oxygen into the skin. The chlorophyll content of the material being cooked gradually decreases. Meanwhile, reduced chlorophyll content is due to the increased activity of the chlorophyllase enzyme, which degrades chlorophyll. The degradation serves as a synthesis material in the manufacture of ethylene. This is consistent with the research of Mudyantini et al. (2017) and Bons et al. (2016) on sapodilla fruit.

Carotenoids

Figure 3 shows carotenoid data for three sweet potato types with a 25-day shelf life at varying chitosan coating amounts and temperatures.

The data showed that storage at 5 °C contained carotenoids higher than other temperature treatments. The activity of the enzymes that play a role in the degradation of pigments is inhibited, and for chitosan 0 g/L, orange tubers contain high beta carotene. The treatment of 15 g/L white tubers effectively maintains

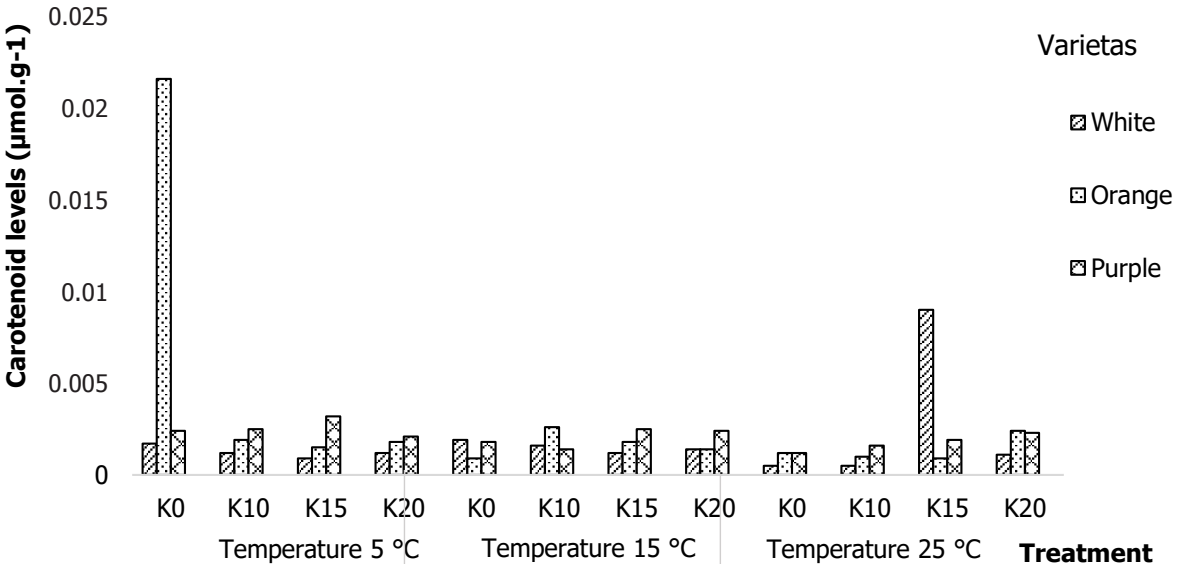

Figure 3. Carotenoids of three varieties of sweet potato aged 25 days shelf life at various concentrations of chitosan coating with variations in storage temperature

chlorophyll and carotenoid levels. Storage at higher temperatures causes faster pigment degradation. Carotenoids are compounds with a high level of unsaturation, and storage temperature conditions can easily destroy these pigments. Even though high carotenoid data were obtained at 5 °C, the tubers showed CI symptoms. Therefore, conditioning may be required to maintain carotenoid levels without compromising degradation due to CI at low temperatures. Li et al. (2018) reported that conditioning for several days at a temperature slightly above the CI threshold before storage at 4 °C could maintain carotenoid degradation.

Reducing Sugar

Reducing sugar data of three sweet potato varieties aged 25 days of shelf life at various concentrations of chitosan coating with temperature is shown in Figure 4.

Based on Figure 4, the reducing sugar content at 15 °C is lowest compared to 5 and 25 °C. At 25 °C, respiration is fast, and carbohydrates are immediately

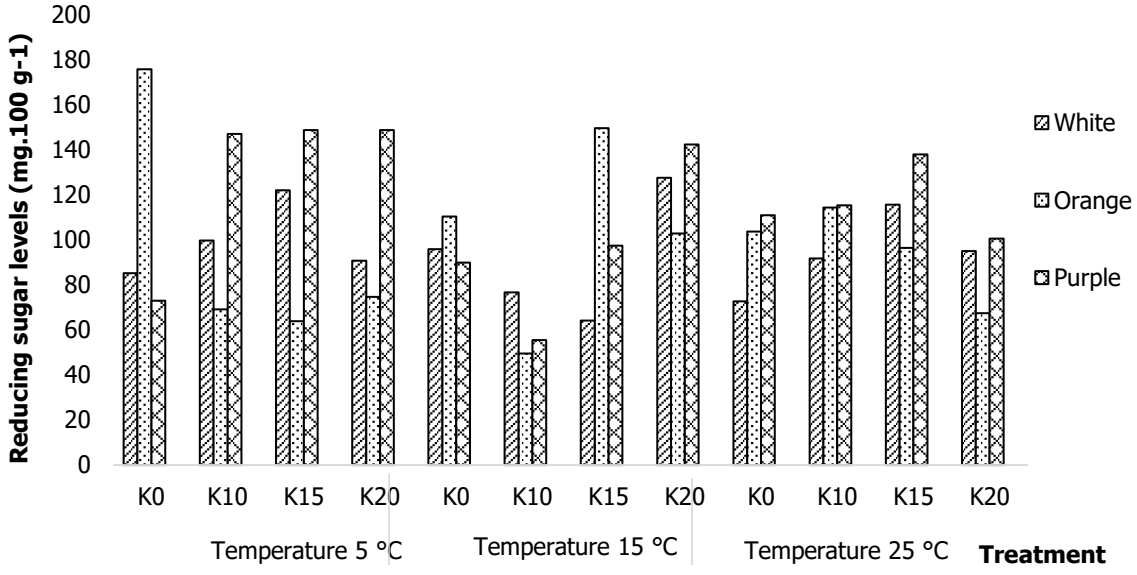

Figure 4. Reducing sugar of three sweet potato varieties aged 25 days shelf life at various concentrations of chitosan coating with variations in storage temperature

hydrolyzed to glucose. Meanwhile, for the temperature of 5 °C, the tubers are damaged because they experience a chilling injury characterized by the ice formation on the skin's surface. This causes the product to contain water when removed from the storage room, hence the hydrolysis process of carbohydrates is high. The orange tuber has a higher reducing sugar level than the other temperatures, presumably because this variety is genetically sweet. At a temperature of 5 °C, the tubers experience a chilling injury. At a temperature of 25 °C, they experience damage, including the emergence of shoots and pests.

Coating with various concentrations of chitosan caused the pores of the tuber skin surface to close. Therefore, the respiration rate was inhibited, causing the accumulation of total reducing sugars. The decrease in reducing sugar levels is due to increased respiration at the glycolysis stage, which is the breakdown of reducing sugars into pyruvic acid and produces CO_2 and H_2O. Chitosan and low-temperature treatment reduced the sugar content of three sweet potato varieties. Furthermore, the concentration of 10 g/L and 0 g/L at the temperature of 15 °C and 5 °C had the lowest and highest reducing sugar value. Therefore, a concentration of 20 g/L is ineffective because the coating is broken.

Hardness

At a shelf life of 25 days at various temperatures and chitosan coatings, three sweet potato varieties showed variations in the decrease in hardness of the skin. Table 1 shows that the hardness at 15 °C is higher than other temperature treatments. At 5 °C of lowest hardness, the tubers become soggy and watery.

The treatment with chitosan showed that the hardness was higher than others at a concentration of 20 g/L and a temperature of 25 °C for all colors of sweet potato tubers. This is because the sweet potato tuber skin is dry and becomes hard due to water loss in the tissue. As for the treatment of chitosan 10 and 15 g/L at 15 °C, the hardness was still good, and the skin was fresh and not dry.

In post-harvest tuber storage, the activity of pectinase enzymes, such as polygalacturonase, will break down pectin into simpler molecules and decrease skin rigidity or hardness. This decrease in hardness is due to the loosening of the cohesive forces between the cell walls forming the outermost layer of the tuber. Furthermore, pectin is the main constituent of the primary wall of plant cells, and the loss of water causes an increase in hardness value. This is appropriate to Shehata et al. (2012) research on cucumber.

The Effect of Thickness of Chitosan Layer on the Surface of Sweet Potato Tuber Skin

The concentration influences the penetration of chitosan through the tuber skin, an epidermal tissue composed of 1 to 3 layers of cells. Chitosan with a more concentration cannot easily penetrate the epidermal layer. Meanwhile, a more dilute concentration makes it easier to enter the deeper layers. Chitosan with a high concentration will be anchored to the skin's surface, making the film layer thicker. The low concentration will insert through the gaps between the cells to form a unit with the tuber skin and only form a thin layer on

Table 1. Hardness of three sweet potato varieties aged 25 days of shelf life at various concentrations of chitosan coating with variations in storage temperature

Temperature (°C)	Variety	Chitosan (g/L)			
		0	10	15	20
5	U	0.50 ± 0.00^{a}	0.50 ± 0.00^{a}	0.50 ± 0.00^{a}	0.50 ± 0.00^{a}
	O	0.50 ± 0.00^{a}	0.50 ± 0.00^{a}	0.50 ± 0.00^{a}	0.50 ± 0.00^{a}
	P	0.50 ± 0.00^{a}	0.50 ± 0.00^{a}	0.50 ± 0.00^{a}	0.50 ± 0.00^{a}
15	U	2.50 ± 0.00^{de}	2.57 ± 0.15^{e}	2.50 ± 0.00^{de}	2.00± 0.00^{c}
	O	3.00 ± 0.00^{g}	3.00 ± 0.00^{g}	2.57 ± 0.6^{e}	2.47 ± 0.06^{de}
	P	2.50 ± 0.00^{de}	2.80 ±0.15^{f}	3.00 ± 0.00^{g}	2.10 ± 0.17^{c}
25	U	2.00 ± 0.00^{c}	2.03 ± 0.06^{c}	1.50 ± 0.00^{b}	2.50 ± 0.00^{de}
	O	1.50 ± 0.00^{b}	2.00 ± 0.00^{c}	2.00 ± 0.00^{c}	3.00 ± 0.00^{g}
	P	2.27 ± 0.06^{d}	2.87 ± 0.15^{f}	2.43 ± 0.11^{d}	3.00 ± 0.00^{g}

Note: Values are means ± standard deviation. Values followed by the different letters in the same column and row indicate significant differences at Duncan Multiple Range Test ($p < 0.05$). U=purple sweet potato, O=orange sweet potato, P=white sweet potato.

the skin's surface. The same information was previously reported by Mudyantini et al. (2017) on coating Fosberg sapodilla fruit (*Manilkara achras* (Mill.) with chitosan. Table 2 presents data on the thickness and penetration of the chitosan layer on the tuber skin of three sweet potato varieties. Based on the Japanese Industrial Standard, the maximum edible film thickness is 0.25 mm. The highest thickness at a concentration of 20 g/L was 18.2 µm (0.0182 mm) which still meet the standard.

Table 2. Thickness and penetration of the chitosan layer on the tuber skin of three sweet potato varieties aged 25 days with various concentrations of chitosan coating and variations in storage temperature (µm)

Chitosan concentration (g/L)	The average thickness of the chitosan layer (µm)	The average penetration into the epidermal cells of the tuber skin (µm)
10	3.2 ± 0.1^{a}	3.2 ± 0.4^{m}
15	7.0 ± 0.35^{b}	2.5 ± 0.1^{l}
20	18.2 ± 0.45^{c}	0.5 ± 0.07^{k}

Note: Values are means ± standard deviation. Values followed by the different letters in the same column and row indicate significant differences at Duncan Multiple Range Test ($p < 0.05$).

Respiration

Respiration data showed that the storage temperature of 5 °C and 25 °C had the lowest and highest oxygen consumption compared to others. Respiration data of three sweet potato varieties for 25 days of shelf life at various concentrations of chitosan coating with temperature are shown in Figure 5.

At low temperatures, the activity of enzymes that play a role in respiration is inhibited. Therefore, the rate of glycolysis and redox will be hampered compared to warmer temperature conditions. The decrease in respiration rate was greatly influenced by the coating of chitosan that closed the tuber skin pores and hindered oxygen diffusion. This is consistent with the research of Kurniawan et al. (2013) on sapodilla fruit coated with chitosan.

Vitamin C

Figure 6 depicts the vitamin C test of three varieties of 25-day-old sweet potatoes with varying chitosan coating amounts and temperatures.

Figure 6 shows that at a temperature of 15 °C, the vitamin C content in tubers is the highest compared to other temperatures, followed by 25 °C and 5 °C. Ascorbic acid is easily oxidized, and the process is fast under alkaline conditions, at high temperatures, and exposure to sunlight. This oxidation will be inhibited when vitamin C is left in an acidic state or stored at low temperatures.

Shelf Life

Table 3 displays the shelf-life data of three sweet potato types treated with varying chitosan coatings and storage temperatures.

The colder the temperature, the longer the shelf life, but the data shows that at 15 °C, it shows the

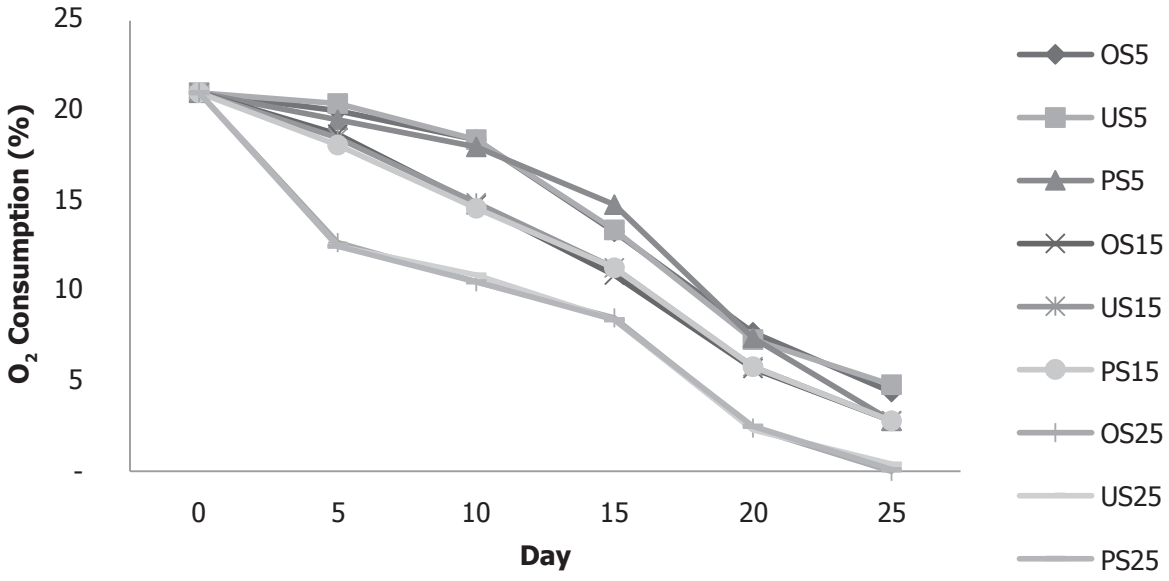

Figure 5. O_2 consumption of three sweet potato varieties aged 25 days of shelf life at various concentrations of chitosan coating with variations in storage temperature

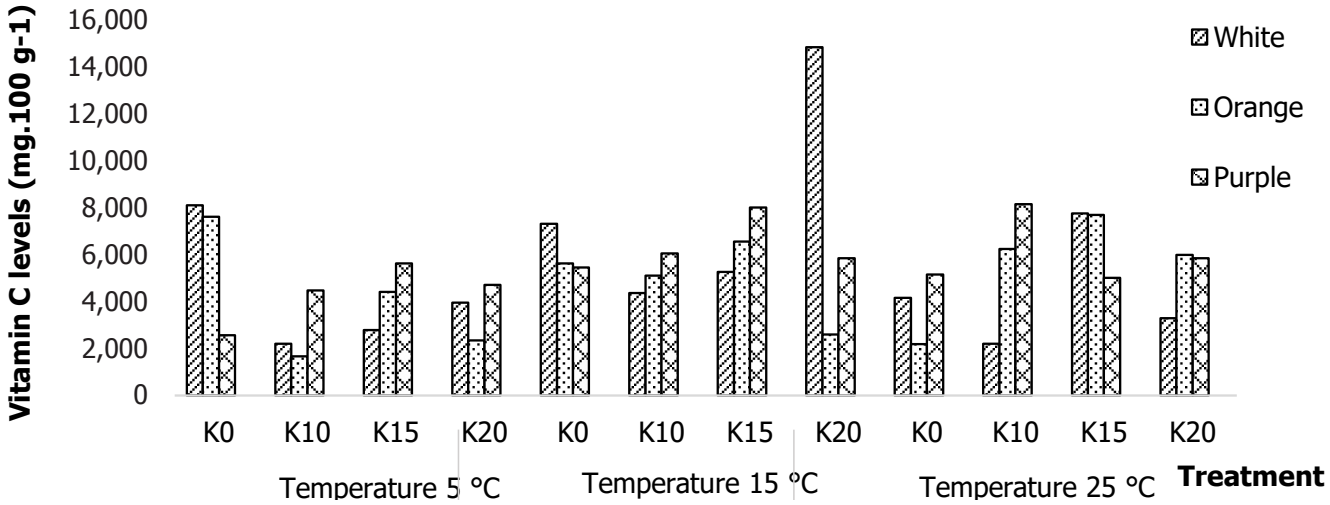

Figure 6. Vitamin C of three sweet potato varieties aged 25 days shelf life at various concentrations of chitosan coating with variations in storage temperature

Table 3. Average shelf life of three sweet potato varieties after being treated with various variations of chitosan coating and storage temperature

Temperature (°C)	Variety	Chitosan (g/L)			
		0	10	15	20
5	U	19.00±0.00^{l}	19.63±0.35^{l}	20.33±0.57^{m}	19.67±0.57^{lm}
	O	16.00±0.00^{i}	17.67±0.57^{jk}	18.33±0.57^{k}	16.33±0.57^{i}
	P	12.00±0.00^{f}	14.33±0.57^{g}	15.33±0.57^{h}	14.00±0.00^{g}
15	U	23.00±0.00^{n}	24.00±0.00^{o}	25.00±0.00^{p}	23.67±0.57^{o}
	O	19.67±0.57^{lm}	20.33±0.57^{m}	20.33±0.57^{m}	19.00±0.00^{l}
	P	16.33±0.57^{i}	17.33±0.57^{j}	18.00±0.00^{k}	16.67±0.57^{i}
25	U	9.00±0.00^{c}	10.00±0.00^{d}	11.00±0.00^{e}	10.33±0.57^{d}
	O	8.00±0.00^{b}	8.00±0.00^{b}	9.00±0.00^{c}	9.00±0.00^{c}
	P	7.00±0.00^{a}	7.00±0.00^{a}	7.00±0.00^{a}	7.00±0.00^{a}

Note: Values are means ± standard deviation. Values followed by the different letters in the same column and row indicate significant differences at Duncan Multiple Range Test ($p < 0.05$). U=purple sweet potato, O= orange sweet potato, P= white sweet potato.

longest shelf life compared to other treatments. At 25 °C and 5 °C, it lasts only 11 and 20 days, and the purple sweet potato had the longest shelf life, followed by the orange and white colors. Differences in varieties cause the levels of secondary metabolites and affect the shelf life. This is consistent with the data on the flavonoid levels in Figure 7. Therefore, cooling the tubers is an effective treatment to reduce the respiration rate. Storage at low temperatures is used to delay maturity and inhibit the expression of genes encoding various hydrolytic enzymes in ethylene biosynthesis (Tacken et al., 2010). Moreover, sweet potato tubers are sensitive to low temperatures (Choundary et al., 2010). This temperature is an effective treatment in -post-harvest handling of tubers, but each type has different sensitivity.

Flavonoid

Based on Figure 11, the flavonoid level of orange and purple tubers is higher than white. Furthermore, orange tubers have a higher flavonoid content than purple. Flavonoids at 5 °C are higher than other temperatures and are secondary metabolites produced when the material is stressed. Figure 7 depicts the flavonoid content of three sweet potato types with a 25-

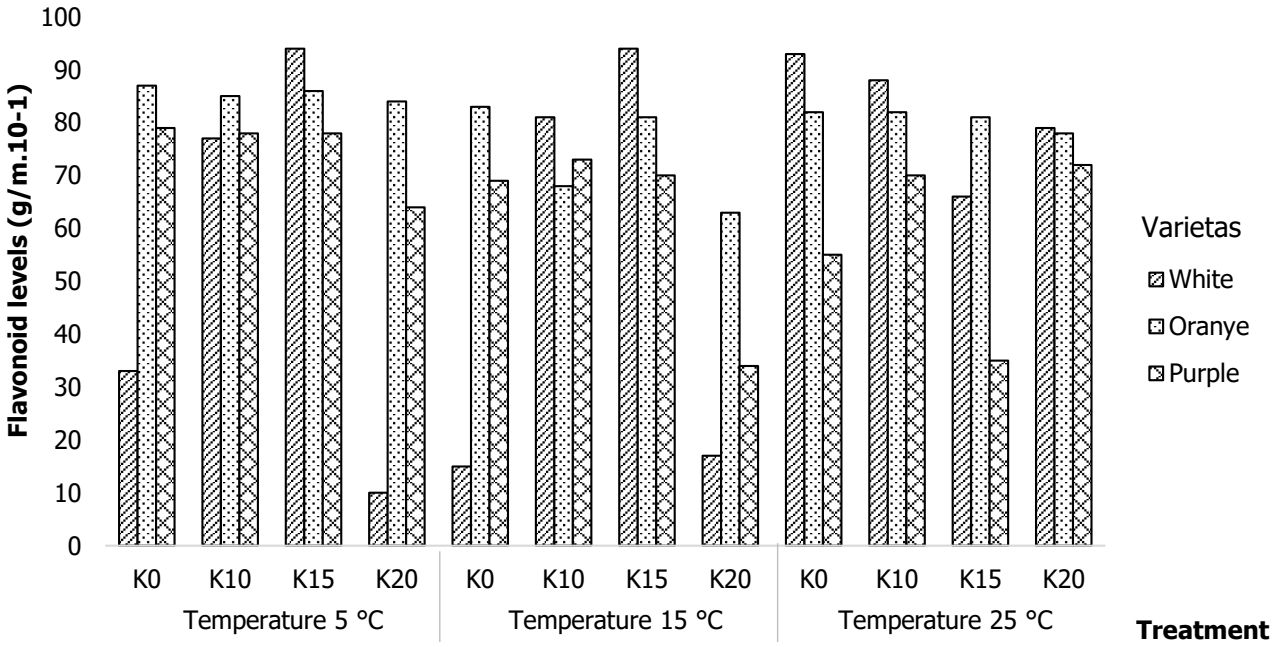

Figure 7. Flavonoids of three sweet potato varieties aged 25 days shelf life at various concentrations of chitosan coating with variations in storage temperature.

day shelf life at varying chitosan coating concentrations and storage temperatures.

Flavanoids are active secondary metabolites compounds widely found in all plants and foods with anti-inflammatory, antiviral, cardioprotective, anti-diabetic, and anti-cancer effects. They are found in plants, which produce the yellow, red, orange, blue, and purple pigments of fruits, flowers, and leaves (Arifin & Ibrahim, 2018).

CONCLUSION

The content of secondary metabolites of sweet potato increased at low or high temperature and excessive chitosan concentration treatments. There were variations in the secondary metabolite levels of the three sweet potato varieties. The best treatment to extend the shelf life of purple sweet potato tuber to 25 days is a 15 g/L chitosan coating with storage at 15 °C while the sweet potato is damaged at 5 °C of storage.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest with other parties.

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