The effect of xanthan-based edible coatings enriched with oleic acid on the storage quality and antioxidant properties of sapodilla (*Manilkara zapota*) fruit

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Abstract

The sapodilla fruit has a limited shelf life due to its perishability and rapid moisture loss. The use of edible coatings has attracted significant interest because they are effective in prolonging the shelf life of fruits. This research study aims to evaluate the effectiveness of an edible coating made from xanthan gum (XG) (0.1% and 0.2%) combined with oleic acid (Ol) (1%) in prolonging the storage duration of sapodilla fruit at a temperature of 8 ± 1 ℃ and a relative humidity (RH) of 85-90%. The treated fruits significantly reduced weight loss, with the minimum weight loss observed in the Xan 0.2% + Ol treatment. Except for the Ol treatment, the other treatments showed a higher level of firmness compared to the control. At the end of the experiment, the treatments significantly reduced fruit respiration. The treated fruits also showed significantly increased antioxidant capacity and higher levels of ascorbic acid compared to the control group. The lowest TSS (22.8%) level was noted in the Xan 0.2 + Ol treatment. Moreover, the results showed that fruit treated with the Xan 0.1% + Ol coating exhibited higher activity levels in the superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzymes compared to fruit treated with the Xan 0.2 + Ol coating and the control samples. In general, Xan 0.2 + Ol and Xan 0.1% + Ol demonstrated the highest overall quality compared to the control and other treatments. Therefore, the use of these treatments is recommended for maintaining the quality of sapodilla fruit.

Keywords: Sapodilla, coatings, Xanthan, oleic acid, storage.

1. Introduction
Sapodilla, *Manilkara zapota*, is the most well-known and the most widely used fruit of the Sapotaceae family. Sapodilla is a rich source of nutrients (carbohydrates, acids, proteins, amino acids), minerals (potassium, calcium, iron), and includes numerous bioactive compounds primarily composed of allergitannins, galotannins, phenolic acids, and flavonoids. Due to its rich phytochemical profile in both edible and non-edible parts, Sapodilla has various potential medicinal applications that can be understood through different biological activities. The fruit of Sapodilla, due to its perishability and quick moisture loss, has a short shelf life, but post-harvest technologies can enhance fruit storage to some extent. There are several methods for preserving and improving the post-harvest life of fruits and vegetables, among which the use of biodegradable films and coatings is very promising (Khalil et al., 2020). Since the edible coating is made from natural materials, it is safe and suitable for human consumption (Paidari et al., 2021). Furthermore, edible coatings have many advantages, including the ability to form a semi-permeable barrier against gases and water vapor, help maintain firmness, add gloss to coated fruits while improving market acceptability, enhance mechanical properties, and prevent the loss of volatile compounds (Galus and Kadzi´nska, 2015). Xanthan gum is an extracellular high molecular weight polysaccharide produced by *Xanthomonas campestris* bacteria and is one of the most important commercial microbial hydrocolloids used in the food industry as a thickening and stabilizing agent (Zheng et al., 2019). Xanthan gum-based edible coating is a long-chain polymeric substance containing polymeric functional groups that exhibit unique properties under specific conditions, which can improve the mechanical properties of biodegradable materials. Xanthan can improve the tensile behavior of starch layers without reducing their water absorption capacity and water vapor permeability (Sapper et al., 2019). Gelatin-carboxymethyl cellulose (CMC) films with xanthan gum showed improved physical and mechanical properties (Nur Hazirah et al., 2016). A composite film of nanocapsules/xanthan gum can prolong the storage of freshly cut apples by reducing the initial respiration rate (Galindo-P´erez et al., 2015). Lipid-based coatings (oleic acid) hinder moisture transfer due to their relatively low polarity. Therefore, a new approach is proposed to increase the shelf life of perishable fruits with minimal processing (Md. Sharif, et al., 2017; Mladenoska, et al., 2012; Karunanayake, et al., 2020; Mitelut, et al., 2021). The content of oleic acid has been demonstrated to serve as an emulsifying agent and a base for preserving fruits (Butar-Butar et al., 2021). In a study by Setianingsih et al. (2023), a combination of palmitic, stearic, and oleic acids was employed in an emulsion, leading to enhanced appearance and prolonged shelf life of orange fruits.

Numerous studies have investigated the effectiveness of coatings in preserving the quality of various fruits during storage. These studies have consistently shown positive results. For instance, in a study conducted by Wani et al. (2021), it was found that the use of Arabic gum, carrageenan, and xanthan gum combined with lemon grass essential oil proved effective in maintaining the quality parameters of strawberries during storage. This coating treatment demonstrated superior results compared to the control group, indicating its
potential for extending the shelf life and preserving the quality of strawberries. In another study, guava fruits were coated with a mixture of Arabic gum (10%), oleic acid (1%), and cinnamon oil (1%). This coating significantly delayed browning development in guava compared to other treatments, while also preserving fruit firmness and reducing weight loss. Additionally, it prevented lipid peroxidation and electrolyte leakage at the end of the storage period, indicating its effectiveness in maintaining fruit quality (Vargas et al., 2006). Previous studies have extensively explored the use of fruit coatings; however, this research introduces an innovative approach by utilizing a combination of xanthan gum and oleic acid to preserve the quality of sapodilla fruits. To the best of our knowledge, there is no previous report on the application of this compound for maintaining the quality of sapodilla fruit. Therefore, this research represents the first investigation into the effects of xanthan gum and its combination with oleic acid on the shelf life of sapodilla.

2. Materials and Methods:

2.1. Fruit Treatment and Edible Coating Preparation:

For this study, mature stage fruits of the Alano variety of Sapodilla (Chico) were harvested from the Minab Agricultural Research Station. Immediately after harvest, the fruits were transported to the physiology laboratory. Healthy and uniform fruits were carefully selected for the experiment. The selected fruits were subjected to various experimental treatments, which included the application of edible coatings comprising xanthan gum at concentrations of 0.1% and 0.2%, along with oleic acid at a concentration of 1.0%. Following the application of the coatings, the treated fruits were stored in a storage at a temperature of 8±1℃ and a relative humidity of 90-85% for 10, 30, 20, and 40 days.

2.2. Weight Loss:

The weight loss of the fruits will be measured using a digital scale by weighing each individual fruit on the first day and at regular intervals (every 10 days). The percentage of weight loss will be calculated using the following formula (Juhaimi et al., 2012).

\[
\text{Weight loss (\%)} = \left(\frac{\text{Fruit initial weight} - \text{fruit weight at each sampling time}}{\text{Fruit initial weight}}\right) \times 100
\]

2-3 - Firmness:

The firmness of the fruits was measured at two points in the middle section (without the peel). Two points on the surface of each fruit were selected and the firmness was reported in N (Juhaimi et al., 2012).

2-4- Respiration:
A specific weight of fruit was placed in a plastic container. The initial CO\(_2\) level (D\(_1\)) was measured during the first instance, and again after 20 minutes (D\(_2\)), using a respirometer device called the STEP Respiratory Sensor. The respiration rate was expressed in mL/kg.h according to the following formula. (Xing et al., 2008).

\[
RCO_2 = (D_2 - D_1) \times 10^6 \times \text{Volume of container} / (\text{Time} \times \text{Fruit weight})
\]

2.5. Total Antioxidant Activity:

The antioxidant activity was calculated based on the method described by Brand-Williams et al. (1995), and the absorbance of the samples was measured using a microplate reader at a wavelength of 517 nanometers. The antioxidant activity was then calculated using the following formula.

\[
\text{Antioxidant activity (\%)} = [1 - (\text{Abs sample} / \text{Abs control})] \times 100
\]

2.6. Soluble Solids Content:

The soluble solids content of the fruit juice was measured in terms of the percent Brix using a digital refractometer (DBR95, Taiwan).

2.7. Ascorbic acid content:

The ascorbic acid content was measured using spectrophotometry and the standard curve of ascorbic acid (O'Grady et al., 2014). The absorbance of the samples was read at a wavelength of 510 nanometers using a microplate reader instrument (Epoch, Bio Tek® Instruments, VT, USA).

2.8. Catalase:

The measurement of catalase enzyme activity in the samples was performed using the method described by Chance and Maehly (1955). The absorbance at a wavelength of 240 nanometers was read using a UV-Visible spectrophotometer model UNICO 2150 for a duration of one minute.

2.9. Ascorbate Peroxidase (APX):

The enzyme activity was measured using the method described by Nakano and Asada (1981). The activity of this enzyme was measured at a wavelength of 290 nanometers for a duration of two minutes using a spectrophotometer. The enzyme activity was then calculated in terms of units per gram of fresh weight (U/g FW min) of the flesh or peel.

2.10. Superoxide Dismutase (SOD):
The SOD enzyme activity was measured using the method described by Giannopolitis and Ries (1977). The absorbance of the samples was read at a wavelength of 560 nanometers using a spectrophotometer. The activity of this enzyme was expressed as units per gram of fresh weight (U/g FW).

3- Results and Discussion

3.1. Weight loss:

The fruits covered with edible coatings exhibited lower weight loss compared to the control throughout the storage. At the end of the storage period, the Xan 0.2 % + Ol treatment resulted in a significant reduction in fruit weight loss by 28.51% compared to the control treatments (Fig. 1). Weight loss is an important indicator of fruit quality during the post-harvest stage and is influenced by transpiration due to differences in vapor pressure between the fruit and the environment (Yaman and Bayonidirli, 2002). It is also influenced by respiration and various physiological mechanisms (Juhami et al., 2012). The application of an edible coating composed of chitosan and essential oils helps minimize water loss in fruits by reducing the rate of water vapor transmission. This coating acts as a barrier, effectively blocking the escape of water vapor and inhibiting excessive transpiration. By regulating the loss of moisture, the coating helps maintain the fruit's water content and prevents dehydration. The reduction in water vapor transmission rate achieved through the coating contributes to the preservation of fruit quality and freshness (Widyastuti et al., 2023). Similar results were obtained in the study conducted by Kumar et al. (2021). They found that a bilayer edible coating consisting of xanthan gum and beeswax on tomato resulted in increased shelf life and improved resistance to water vapor transmission.
Fig. 1 - The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid 1 %, and Xanthan 0.2 % + Oleic acid 1 %) and storage periods on the weight loss of sapodilla fruit during four periods (10 days) of storage at a temperature of 1 ± 8 degrees Celsius and 85% relative humidity (RH). The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

3.2. Firmness

Fruit firmness gradually decreased during storage (Fig. 2-A). The highest level of firmness was observed in the Xan 0.2 % + Ol and Xan 0.1 % + Ol treatments, respectively (Fig. 2-B). Fruit firmness is an important parameter in fresh horticultural products, and it decreases as the storage time increases. The loss of fruit firmness is concurrent with changes that occur in the cell wall structure. Pectin substances are responsible for the integrity of fruits. They are the main components of the middle lamella and predominantly form the initial cell wall structure. The effects of coatings on fruits and their storage conditions vary significantly, as evidenced by the considerable impact on fruit firmness. In the case of sapota fruits, the reduction in firmness can be attributed to several factors, including a decrease in cellular turgor pressure, the release of extracellular and vascular air, and the degradation and breakdown of cell walls (Shah et al., 2016). The addition of lipids to the polymer composition can increase the water repellency behavior of the coating and consequently reduce water permeability. Typically, the permeability values increase linearly with a decrease in the concentration of the essence (Sánchez-González et al., 2010). In this regard, Vargas et al. (2006) reported that the combination of oleic acid with chitosan coating resulted in decreased permeability and respiration rate due to surface solid density.
Fig 2 - The effect of storage periods and different coatings (Control, Xanthan 0.1%, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) on the firmness of sapodilla fruit during four periods (10 days) of storage at a temperature of 1 ± 8 degrees Celsius and 85% relative humidity (RH). The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

3.3. Respiration

As shown in Fig. 3, the fruits treated with Xan 0.2 % + Ol and Xan 0.1 % + Ol showed the lowest respiration rate compared to the other samples. Edible coatings could modify gas transfer (carbon dioxide, oxygen, and ethylene) and consequently delay respiration rate and physiological processes, thus extending the shelf life of fruits and vegetables. Furthermore, previous studies have shown that the delay in respiration rate in fruits can be attributed to the inhibition of ethylene production (Hassan et al., 2018). The results of this study were consistent with the findings reported by Navid et al. (2024), who documented a significant reduction in the respiration rate of jujube fruits covered with xanthan gum coating.

Fig. 3 - The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) and storage periods on the respiration rate of
sapodilla fruit during four periods (10 days) of storage at a temperature of 1 ± 8 degrees Celsius and 85% relative humidity (RH). The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

3.4. Total Antioxidant Activity

The fruits treated with Xan 0.1% + Ol exhibited the highest antioxidant activity, which was 5.23 times greater than the activity observed in the control samples (Fig. 4). Usually, the production of reactive oxygen species (ROS) increases during fruit ripening and storage, leading to oxidative stress and fruit decay. Reports have shown that an increase in total phenolic content correlates with an increase in antioxidant capacity (Etemadipoor et al., 2020). In this study, since the combined xanthan coating with oleic acid disrupts the ripening process, it results in higher antioxidant activity in the fruit. Additionally, several changes in vitamin content throughout the ripening process can influence antioxidant activity.

![Graph showing the interaction effect between treatments and storage periods on total antioxidant activity.](image)

**Fig. 4** - The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) and storage periods on the total antioxidant activity of sapodilla fruit during four periods (10 days) of storage at a temperature of 1 ± 8 degrees Celsius and 85% relative humidity (RH). The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

3.5. Ascorbic acid content

The highest content of ascorbic acid was observed in the fruit treated with Xan 0.1% + Ol (137.86 mg/100 g FW) compared to the control (100.41 mg/100 g FW) (Fig.5). Ascorbic acid acts as an antioxidant in fruits and reduces fruit damage. This action is achieved through the elimination of free radicals produced during the ripening and oxidation process. The presence of oxygen can have a negative impact on the ascorbic acid content in fruits (Ayranci and Tunc, 2004). The presence of an edible coating on the fruit reduces the
detrimental effects of oxygen, and this is accomplished through the performance of antioxidant compounds present in the coating and their role as a barrier against oxygen transfer (Oliveira et al., 2017). Therefore, the combined xanthan coating with oleic acid enabled the fruits to retain higher levels of ascorbic acid compared to other treatments. This was accompanied by reduced oxygen permeability and limited moisture transfer from the fruit surface. In a similar report, Kumar et al. (2023) reported that an edible coating based on xanthan gum and pomegranate peel extract on mango fruit can enhance the physical and antioxidant properties of the fruit due to the increased flexibility it provides.

Fig. 5 - The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) and storage periods on the ascorbic acid content of sapodilla fruit during four periods (10 days) of storage at a temperature of 1 ± 8 degrees Celsius and 85% relative humidity (RH). The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

3.6. Total soluble solids and TSS/TA Ratio

The highest increase in TSS (27.2 %) was observed in the control fruit at the end of storage, while the lowest increase (22.80 %) was observed in fruits coated with Xan 0.2 % + Ol (Fig. 6-A). Generally, the TSS of fruits gradually accumulates during ripening. This phenomenon may be due to the hydrolysis
reaction and conversion of starch into simple sugars. At the end of storage, the control fruit showed an increase in the TSS/TA ratio by 104.099 compared to its initial state, while the Xan 0.1 % + Ol coating resulted in a decrease in the TSS/TA ratio (Fig. 6-B). The TSS/TA ratio gradually increased during storage (Fig 6-C). Etamadi et al. (2020) reported similar results on coated guava fruits with a combination of Arabic gum, oleic acid, and cinnamon essential oil.
Fig. 6 - The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) and storage periods on the total soluble solids (TSS) (A), and TSS/TA ratio (B-C) of sapodilla fruit during four periods (10 days) of storage at a temperature of 1 ± 8 degrees Celsius and 85% relative humidity (RH). The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

3.7. Enzymatic activity

The activity of the CAT enzyme showed a significant increase within the first 20 days of storage. The highest enzyme activity was observed in fruits treated with a combination of 0.1% Xan + Ol during the second storage period (Fig. 7-A). Similar findings have been reported for other fruits such as chickoo (Camargo et al., 2016) and lychee (Zhang et al., 2018). The peak increase in APX activity was observed on the tenth day in the Xan 0.1 % + Ol treatment, followed by a decrease (Fig. 7-B). Our findings are consistent with a previous study by Ali et al. (2021), which found that using a CMC coating prior to storage preserved higher enzymatic activities and deactivated free radicals, reducing senescence in 'Kinnow' mandarin fruit under low-temperature conditions. The maximum activity of SOD enzyme in the coated fruits was observed 40 days after storage, and the peak in the Xan 0.1 % + Ol-coated fruits was significantly higher than the control group (P < 0.05) (Fig. 7-C). These results are like the study conducted by Yuan et al. (2023), which examined the effect of a combined treatment of 1-methylcyclopropene and melatonin on the quality characteristics and active oxygen metabolism of stored mango fruit. An oxidative stress occurred throughout the entire storage period, characterized by an increase in active oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radicals. The antioxidant system, which includes enzymes such as POD, SOD, CAT, and APX, plays an important role in preventing or reducing damage caused by ROS (Wang & Gao, 2013).
Fig. 7 - The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) and storage periods on catalase (A), ascorbate peroxidase (B), and superoxide dismutase (C) activities of sapodilla fruit during four periods (10 days) of storage at a temperature of 1 ± 8 degrees Celsius and 85% relative humidity (RH). The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

Conclusion:

In conclusion, this study demonstrated that the application of edible coatings made from xanthan gum (XG) at concentrations of 0.1% and 0.2%, combined with oleic acid (Ol) at a concentration of 1%, effectively prolonged the storage duration of sapodilla fruit. The treated fruits exhibited reduced weight loss, with the Xan 0.2% + Ol treatment showing the minimum weight loss. Additionally, the treated fruits maintained higher firmness levels compared to the control group, except for the Ol treatment. The coatings also significantly reduced fruit respiration and enhanced antioxidant capacity, as well as increased levels of ascorbic acid. The Xan 0.2% + Ol treatment resulted in the lowest total soluble solids (TSS) level. Furthermore, the Xan 0.1% + Ol coating demonstrated higher activity levels in the superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzymes compared to the Xan 0.2% + Ol coating and the control. General, the Xan 0.2% + Ol and Xan 0.1% + Ol treatments exhibited the highest overall quality among the tested treatments. Given the challenges associated with postharvest preservation of this fruit, the use of these coatings presents a suitable method for extending the storage period and maintaining the quality.

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