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# The Effect of Concentration and Immersion Time of Sodium Hydrogen Sulfites as an Anti-Browning Agent in Sliced Apples (*Malus sylvestris Mill.*)

*N.A. Utama*[*,1], *C.D. Nusivera*[1], *I.Prabasari*[1], *C.K. Setiawan*[1]

[1] *Department of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia*

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**CORRESPONDING AUTHOR**

*E-mail: nafi@umy.ac.id

**A B S T R A C T**

Sodium hydrogen sulfite or sodium bisulfite (NaHSO3) is a common anti-browning agent to preserve fresh produce. The color of fruits and vegetables can be maintained by inhibiting certain enzymes involved in the browning process. The study evaluates different concentrations (50, 100, 150, and 200 ppm) and immersion time (three and five minutes) of NaHSO3 applied to fresh-cut apples. Sliced apples (n=189 slices) were grouped into nine groups: N50I3 (50 ppm NaHSO3 immersion for three minutes), N100I3 (100 ppm NaHSO3 immersion for three minutes), N150I3 (150 ppm NaHSO3 immersion for three minutes), N200I3 (200 ppm NaHSO3 immersion for three minutes), N50I5 (50 ppm $NaHSO_3$ immersion for five minutes), N100I5 (100 ppm NaHSO3 immersion for five minutes), N150I5 (150 ppm NaHSO3 immersion for five minutes), N200I5 (200 ppm NaHSO3 immersion for five minutes) and control group. The samples were compared between groups for peroxidase activity (unit/min), polyphenol oxidase activity (unit/min), phenolic content (ppm), total antioxidant activity (%), and discoloration (hue) every three days for 15 days. The results showed that immersing freshly cut fruit of Manalagi apples in NaHSO3 50 ppm for three minutes was the best treatment for inhibiting the enzymatic browning process compared to other treatments.

**Contribution to Sustainable Development Goals (SDGs):**
**SDG 2:** Zero Hunger
**SDG 3**: Good Health and Well-being
**SDG 12:** Responsible Consumption and Production
**SDG 5:** Industry, Innovation and Infrastructure

## 1. INTRODUCTION

### *1.1. Research Background*

Apples (*Malus sylvestris* Mill.) are one of the subtropical plants cultivated in Indonesia. The high rate of imported apples implies that local varieties cannot compete with imported apples in attracting consumers. One of the efforts to increase consumer interest in purchasing or consuming local apples is product diversification, such as minimally processed local apples.

The addition of other ingredients did not count as minimal processing. Minimally processed fruits are commonly cleaned, peeled, sliced, and stored at low temperatures, resulting in ready-to-eat fruits with negligible loss of freshness and nutritional value. Various treatments for fresh-cut fruit can disrupt the integrity of its tissues and cells, leading to increased ethylene production, respiration rate, membrane degradation, water loss, and microbial damage. Browning in fruits is undesirable as it can diminish the intention to purchase from prospective consumers.

The current study evaluates the use of sodium bisulfite ($NaHSO_3$) in sliced apples to prevent enzymatic browning. As a sulfiting agent, $NaHSO_3$ is widely used as an additive to delay the browning of fruits and vegetables during processing. The effectiveness and optimum concentration of sodium bisulfite ($NaHSO_3$) in inhibiting enzymatic browning of fresh-cut apples of a local variety (Manalagi var.) to suppress enzymatic browning were determined.

### *1.2. Literature Review*

According to the Indonesian Central Statistics Agency, the national average consumption of apples per household displays an increase between 2021 (1.68%) and 2022 (1.71%) [1]. However, the Ministry of Industry of the Republic of Indonesia reported that the trend of imported fresh apples rose from 2012 to 2016, with an incline of 6.57% [2]. The concentration and time of soaking fresh-cut Manalagi apple fruit in sodium bisulfite reduces the fruit flesh's browning rate. The findings of this study are in line with the research results of Cortez-Vega et al. [3], which stated that the brightness level of fresh-cut apple flesh was highest in the treatment using sodium metabisulfite and indicated that this treatment was the most efficient treatment for inhibiting enzymatic browning.

Minimal processing in foods includes the removal of unnecessary parts, drying, crushing, grinding, fractioning, filtering, roasting, boiling, pasteurization, refrigeration, freezing, repackaging, vacuum packaging, or non-alcoholic fermentation of food in its natural states [4]. Nevertheless, minimally processed fruits are more susceptible to shorter shelf life than fresh fruits due to removing the outer layers [5], [6]. One major concern in fresh-cut fruits is the occurrence of enzymatic browning, which can shorten the shelf life and decrease the quality of fruits [7]. Browning in fruits can be caused by the formation of brown melanin when fruits are exposed to air (i.e., mechanical or microbiological damages) due to the oxidation of phenolic compounds [8]. Enzymes in fruits, such as polyphenol oxidase (PPO) and peroxidase (POD), are responsible for the enzymatic browning of fruits in the presence of oxygen and phenolic compounds [9].

The reaction of enzymatic browning can be avoided using several methods, including deactivating the enzyme or adding an anti-browning agent to disrupt the contact between the enzyme and the substrate [10]. PPO deactivation can be achieved by inhibiting the browning reaction mechanism by eliminating oxygen availability [11] or degrading the protein enzymes of PPO [12]. However, excessive use of sulfite compounds is prohibited by WHO as it can lead to adverse effects similar to allergy reactions, including dermatitis, urticaria, abdominal pain, and rhinitis [13]. The limit of $NaHSO_3$ daily intake as food additives in Indonesia was 0–0.7 mg/kg body weight [14].

### *1.3. Research Objective*

The research aims to determine the effectiveness and optimum concentration of sodium bisulfite ($NaHSO_3$) in inhibiting enzymatic browning of fresh-cut apples of a local variety (Manalagi var.) to suppress enzymatic browning

## 2. MATERIALS AND METHODS

### *2.1. Research design*

The research design of this study employed a Completely Randomized Design with a single-factor treatment. Various concentrations of sodium bisulfite (50, 100, 150, and 200 ppm) and immersion times (three and five minutes) were applied to the research samples. Different combinations of various concentrations and immersion durations resulted in a total of eight treatment groups and one control group.

### *2.2. Sample preparation*

Apples were harvested from an apple orchard in Batu City, Malang Regency (7.829° S, 112.525° E) with the criteria of having an age 114 days after flowering (DAF) with no visible deformation or injuries and weighing between 0.2 – 0.5 kg. The harvested apples were cleaned by dipping them in a chlorine solution (200 ppm) and thoroughly scrubbing the peels with hands. The apples were air-dried before they were cut into six similar pieces, and the core was removed.

### *2.3. The preparation and application of sodium bisulfite*

The stock solution with a concentration of 200 ppm was prepared by adding 1.6 g of sodium bisulfite to eight liters of water. After that, the solution was mixed until homogenized, and a dilution process was carried out to make solutions with concentrations of 150 ppm, 100 ppm, and 50 ppm. The fruit slices were immediately dipped into the sodium bisulfite solution, according to the subsequent treatment groups. The fruit pieces were packed and stored in the refrigerator at 5°C for 15 days.

### *2.4. Measured parameters*

#### *2.4.1. Color*

Brightness was measured based on color intensity using a Minolta CR - 400 Chromameter. Observations were made every three days for 15 days. Color measurements include CIELAB color. The L* value represents brightness with a value of 0 (dark/black) to 100 (light/white), while a and b are chromameter coordinates, where a* implies the color green (negative) to red (positive) and b* indicates the color blue (negative) to yellow (positive). The total color change (ΔE) during storage is obtained using the formula:

$$\Delta E = \frac{[(\Delta L) \times 2 + (\Delta a) \times 2 + (\Delta b) \times 2]}{2}$$

Description :
ΔE = Total color difference
ΔL (L sample - L standard) = Difference between light and dark (+ = lighter, - = darker)
Δa (a sample - a standard) = Difference between red and green (+ = red, - = green)
Δb (b sample - b standard) = difference between yellow and blue (+= yellow, - = blue)

#### *2.4.2. Polyphenol oxidase activity*

The PPO enzyme activity test was conducted by grinding five grams of the sample and mixing it with 115 ml phosphate buffer. The mixture was then filtered and transferred into a 24 ml Erlenmeyer flask. Subsequently, 1 ml of $H_2O_2$ and 2.5 ml of Guaiacol were added. Five mL of the mixture was transferred into a test tube and subjected to centrifugation at 2680 rpm for 30 minutes. The solution was measured using a spectrophotometer at a wavelength of 470 nm.

#### *2.4.3. Phenolic compound content*

Total phenol testing was done by mashing 1 gram of the sample in a mortar and adding 10 ml of distilled water. Then, 0.5 ml of the sample was put into a test tube and diluted using 5 ml of

distilled water. After five minutes, 1.5 ml $Na_2CO_3$ and 1.5 ml Folin reagent were added. The solution was shaken until it changed color to blue. The solution was observed using a spectrophotometer with a wavelength of 765 nm.

#### *2.4.4. Total antioxidant activity*

The total antioxidant activity (TAA) test was carried out by mashing one gram of the sample and diluting it in 10 ml of methanol. One ml sample was taken into a test tube and combined with 1 ml DPPH solution. The mixture was left undisturbed for 30 minutes, after which 3 ml of methanol was added. The resulting solution was analyzed using a spectrophotometer set at a wavelength of 517 nm.

### *2.5. Statistical analysis*

The data obtained were analyzed using Analysis of Variant (ANOVA) at a significance level of 5%. If significant differences exist between the treatments tested, further tests are carried out using Duncan's Multiple Range Test (DMRT).

## 3. RESULT AND DISCUSSION

### *3.1. Color*

The treatment of various sodium bisulfite concentrations and immersion time of fresh-cut Manalagi displayed significant differences in the color of apples on observation days 0, 3, and 9 (Figure 1).

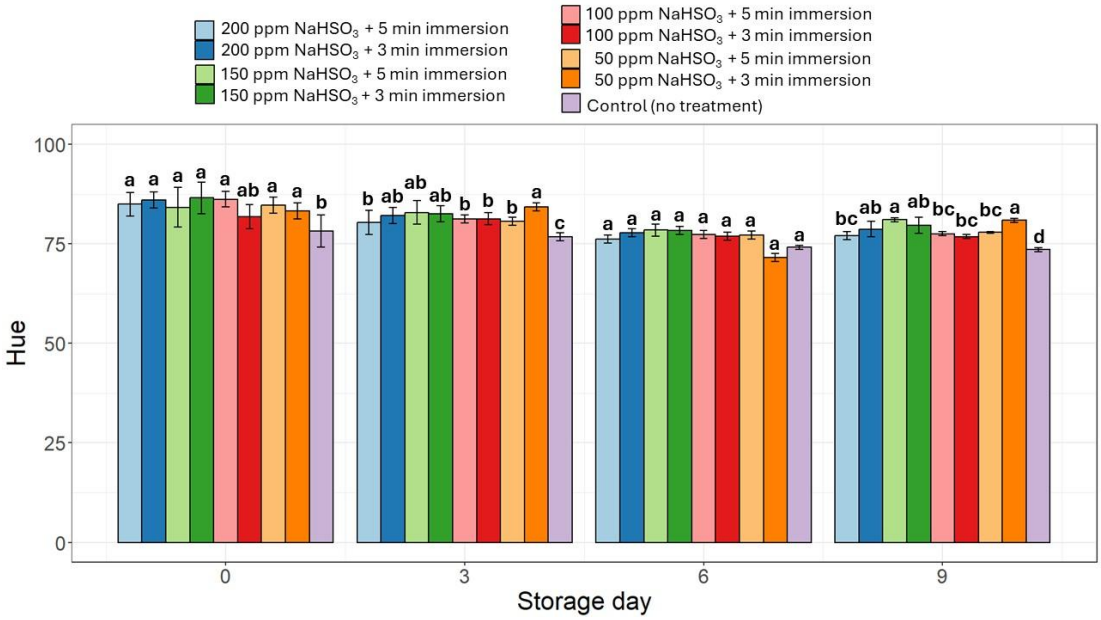


**Fig. 1.** Color measurement (hue) result of sliced apples immersed in different sodium bisulfite concentrations and duration after nine days of storage. The values with the same alphabetic letters and orders were not significantly different according to Duncan's Multiple Range Test (DMRT) with a 5% significance level.

The control samples displayed the lowest hue value compared to other treatment groups on days 0, 3, and 9. On the other hand, samples with the least concentration of $NaHSO_3$ and the shortest duration of immersion resulted in the significantly highest hue score (80.90 ± 0.51) on the last day of observation. However, the sapodilla fruits treated with 150 ppm $NaHSO_3$ solution for five minutes were not significantly different from the highest hue value treatment group (81.05 ± 0.58).

A previous study reported that the reaction between sulfite and quinone, and soaking with bisulfite solution, effectively inhibited the appearance of brown color on fruit and vegetables [15]

On the ninth day, all treatments of fresh-cut Manalagi apples in sodium bisulfite experienced an increase in the brightness of the fruit flesh. The effect of sodium bisulfite on fruit color is thought to be due to the ability of the sulfite compound to reduce the oxidation products of quinone to the previous colorless phenolic form.

Soaking fresh-cut Manalagi apple fruit in sodium bisulfite with a concentration of 50 ppm for 3 minutes and 150 ppm for 5 minutes resulted in a brighter fruit flesh color than other treatments on the ninth day. Meanwhile, soaking in sodium bisulfite at a concentration of 200 ppm for 5 minutes produced the darkest fruit flesh color compared to other treatments. Soaking fruit using sodium bisulfite can have a positive effect on the brightness of the fruit flesh if the conditions match the saturation capacity of the fruit flesh because too much residue in the fruit flesh as a result of a solution with too high a concentration can trigger a decrease in the brightness level of the fruit flesh [16]. Sulfite residue is the remaining sulfite left in a food ingredient. Sulfite residue is caused by soaking and water-soluble sulfites entering the fruit tissue.

### *3.2. Polyphenol oxidase activity*

Changes in PPO enzyme activity in fresh-cut Manalagi apples after soaking in natrum bisulfite are presented in Figure 2.


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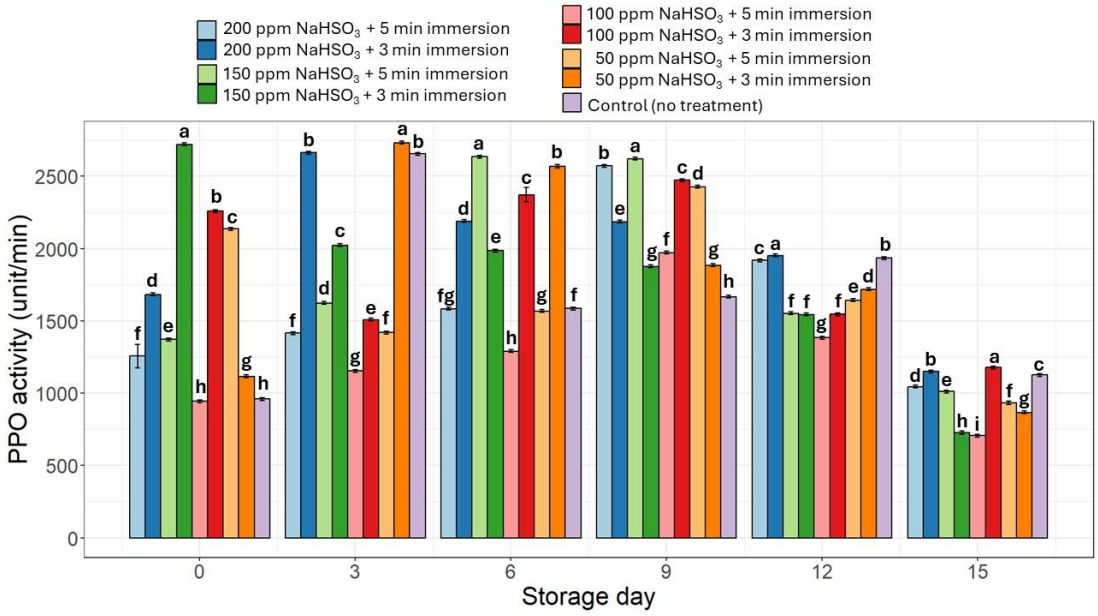


**Fig. 2.** Polyphenol oxidase (PPO) activity of sliced apples immersed in different sodium bisulfite concentrations and duration after 15 days of storage. The values with the same alphabetic letters and orders were not significantly different according to Duncan's Multiple Range Test (DMRT) with a 5% significance level.

Different concentrations and immersion times can lead to varied inhibition effects of PPO activity. The treatment of dipping in a 100 ppm $NaHSO_3$ solution for five minutes showed the lowest PPO activity on all observation days except day 6. The decrease in PPO enzyme activity was caused by soaking fresh-cut Manalagi apples using sodium bisulfite, which effectively inhibited or reduced enzyme activity. On the 15$^{th}$ day, PPO enzyme activity continued but in lower amounts compared to the previous day, with the lowest activity seen in the treatment of 100 ppm $NaHSO_3$ for five minutes (706.01 ± 10.13 unit/min). Interestingly, the same concentration of $NaHSO_3$ for three minutes results in the highest activity of PPO (1178.33 ± 11.53 units/min).

According to Kuijpers et al. [17], the mechanism for inhibiting browning by sulfite compounds is divided into three ways, namely inhibition of the reaction in the direction of PPO, reduction of o-quinone, thereby reversing the direction of the enzymatic reaction and formation of additional products between sulfite and o-quinone5 preventing further browning activity [18].

Yu Shen et al. [18] stated that sulfite contributes to oxygen reduction, so oxidase cannot oxidize polyphenol constituents or combine with quinone. The inhibition of polyphenol-quinone bond formation causes the quinone to fail to undergo a reaction, and the quinone returns to its previous phenolic form. The activity of the PPO enzyme is very dependent on oxygen concentration because oxygen will convert phenol compounds into brown melanin [19].

On the 15th day, PPO enzyme activity continued but in lower amounts than the previous day. According to Sayavedra-Soto and Montgomery (2007), the sulfite compounds contained in sodium bisulfite cannot completely stop the browning reaction but can only slow down the browning reaction by reducing the activity of the PPO enzyme [20]. Sulfite compounds inhibit the formation of brown pigment by converting o-quinone into colorless sulfo-phenolate, and the inhibition occurs depending on tyrosinase activity [17].

### *3.3. Peroxidase activity*

The two main enzymes responsible for undesirable enzymatic browning in fruits are polyphenol oxidase (PPO) and peroxidase (POD) enzymes. The POD enzyme activity of sapodilla fruits applied with various $NaHSO_3$ concentrations and immersion time is illustrated in Figure 3.

The findings showed similar results to those of the analysis of PPO activity. The inhibition of POD activity was the highest in sapodilla after being soaked with 100 ppm $NaHSO_3$ solution for five minutes on the last day of observation, as indicated by the lowest POD activity (19.27 ± 0.46 unit/min). Furthermore, similar to the PPO activity, the same concentration of $NaHSO_3$ in a shorter time has the highest activity of POD (58.42 ± 0.39 unit/min) compared to other treatment groups. The treatment of 100 ppm $NaHSO_3$ solution with an immersion time of five minutes resulted in the lowest POD activity on all observation days.

The peroxidase enzyme found in fruit and vegetables is a ferriprotoporphyrin (protohemin) as a prosthetic group, an enzyme containing Fe, which will be brown if purified. The bonds between prosthetic groups containing iron and proteins can be stabilized with bisulfate [21]. Sulfite compounds form a complex with iron peroxidase, which can stabilize or even reduce the activity of this enzyme so that the browning reaction in fruit can be inhibited. Li et al. [22] stated that there is a correlation between the brownish color change in fruit during storage and an increase in POD enzyme activity. Peroxidase enzymes are a group of oxidoreductase enzymes capable of catalyzing oxidation reactions by hydrogen peroxide ($H_2O_2$) of several substrates, which are hydrogen donors such as phenol. Peroxidase enzymes in living organisms can catalyze their substrate compounds, while $H_2O_2$ initiates the biosynthesis of several secondary metabolites needed in the growth process. Oxidation of phenolics by peroxidase enzymes with the substrate $H_2O_2$ results in an oxidative coupling reaction, forming phenolic polymers. Oxidation carried out by the peroxidase enzyme on phenolic

compounds causes the formation of a phenoxy radical, where this radical can resonate with the ortho and para positions on the aromatic ring and will then combine with other phenoxy radicals to form new polyphenol compounds.

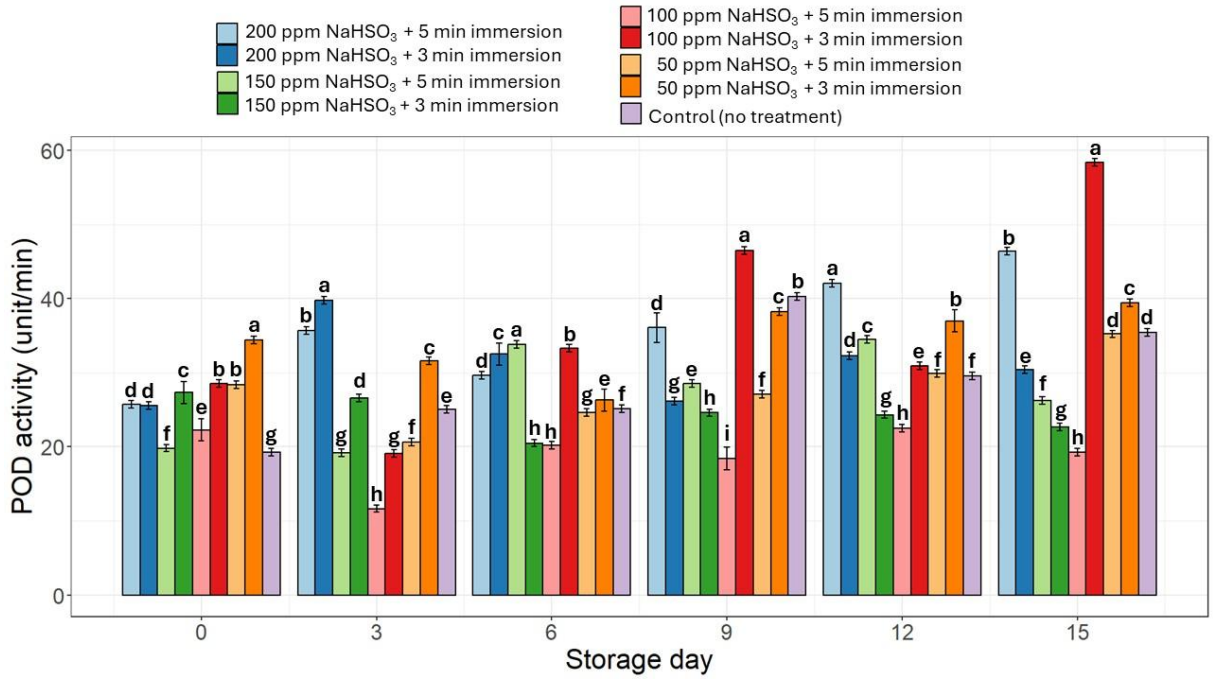


**Fig. 3.** Peroxidase activity of sliced apples immersed in different sodium bisulfite concentrations and duration after 15 days of storage. The values with the same alphabetic letters and orders were not significantly different according to Duncan's Multiple Range Test (DMRT) with a 5% significance level.

### *3.4. Total phenolic content*

Total phenols in fresh-cut Manalagi apples treated with sodium bisulfite soaking showed significantly different results from those without soaking. The dynamics of changes in total phenol in all treatments are displayed in Figure 4.

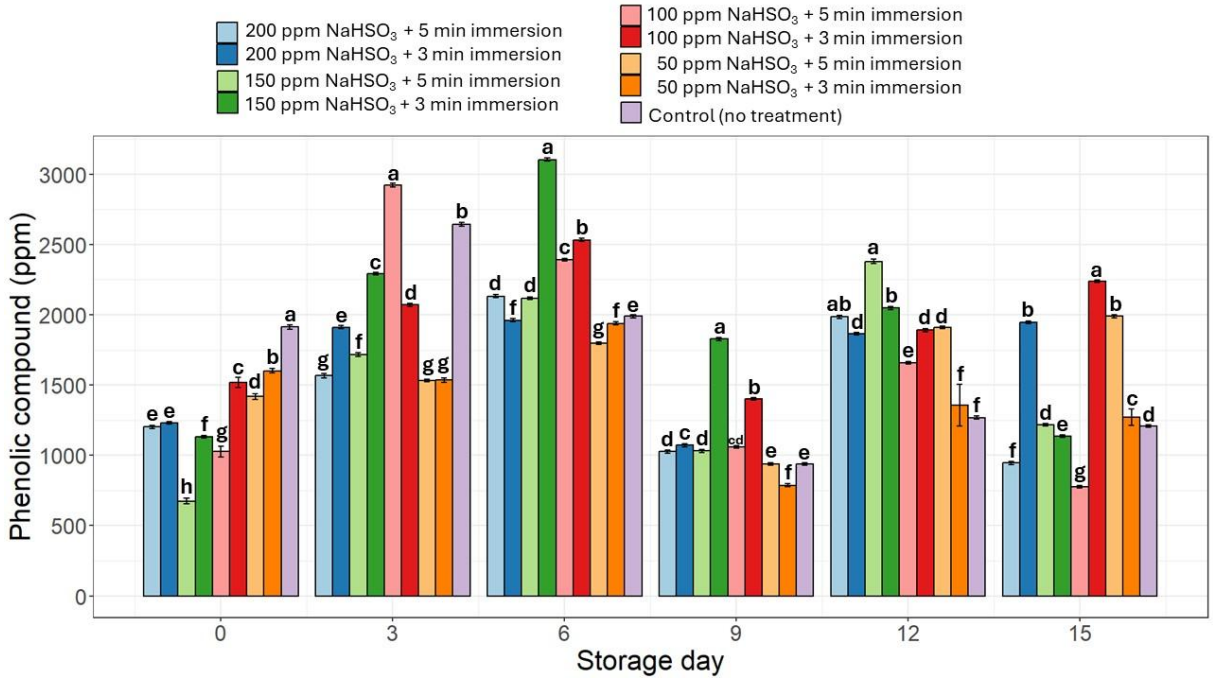


**Fig. 4.** The total phenolic content of sliced apples immersed in different sodium bisulfite concentrations and duration after 15 days of storage. The values with the same alphabetic letters and orders were not significantly different according to Duncan's Multiple Range Test (DMRT) with a 5% significance level.

The soaking treatment in 100 ppm sodium bisulfite for 5 minutes showed the lowest total phenol at the end of the observation compared to other treatments on day 15. Figure 4 illustrates that the phenol content in all treatments tended to

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increase on days three and six, followed by a declining trend on day 9. Moreover, the samples immersed for three minutes in 100 ppm $NaHSO_3$ were significantly higher than the treatment of the same concentration of $NaHSO_3$ for five minutes. The same treatment results in the highest amount of phenolic compounds on the last observation day compared to other groups.

Figure 4 shows that the phenol content in all treatments tended to increase on days 3 and 6, then decreased on day 9. The increase in phenol content until day six was related to the presence of the phenylalanine-ammonia lyase (PAL) enzyme, which also increased due to injury. The phenomenon of total phenol reaching a peak and then decreasing the following day occurs after the PAL enzyme has previously reached its peak due to injury. Total phenol does not increase in parallel with the PAL enzyme, but there is a lag when total phenol reaches its peak. The phenol content increased again on the 12th day and tended to decrease on the 15th day. At the end of the observation, the lowest phenol content was found in samples soaked in sodium bisulfite treatment with a concentration of 100 ppm for 5 minutes.

### *3.5. Total antioxidant activity*

The results of variance analysis showed that dipping fresh-cut Manalagi apples in sodium bisulfite solution with concentrations of 200 ppm, 150 ppm, 100 ppm, and 50 ppm for 3 minutes and 5 minutes showed significant results on the Total Antioxidant Activity (TAA) of fresh-cut Manalagi apples. Total antioxidant activity (TAA) in fresh-cut Manalagi apples during storage can be seen in Figure 5, which shows the TAA trend from day 0 to day 15.

Figure 5 shows that the TAA of fresh-cut Manalagi apples in all soaking treatments fluctuated during storage but tended to be constant from day 3 to day 15. Fresh-cut Manalagi apple fruit without treatment experienced antioxidant activity that continued to decrease from day 3 to day 15. Antioxidant activity in fresh-cut fruit treated with $NaHSO_3$ solution soaking tended to increase on the third day, except for the treatment of 50 ppm $NaHSO_3$ soaking for 3 minutes, which experienced a drastic decrease in TAA but increased again on the sixth day. On the last observation day, samples treated with 50 ppm $NaHSO_3$ solution for three minutes displayed the highest TAA.

This increase in antioxidant activity is related to the number of hydroxyl groups in the sample. It is suspected that sulfite compounds that enter fruit tissue can maintain the content of hydroxyl groups in phenolic compounds so that the capture or neutralization of free radicals continues, such as neutralizing reactive oxygen species (ROS), which can cause DNA damage and accelerate aging. According to Aji [23], the greater the number of hydroxyl groups a phenol compound has, the greater the antioxidant activity produced. This phenol compound can donate hydrogen atoms to reduce DPPH radicals to a more stable form. The number and position of phenolic hydrogen in the molecule influence the free radical scavenging activity of phenolic compounds.

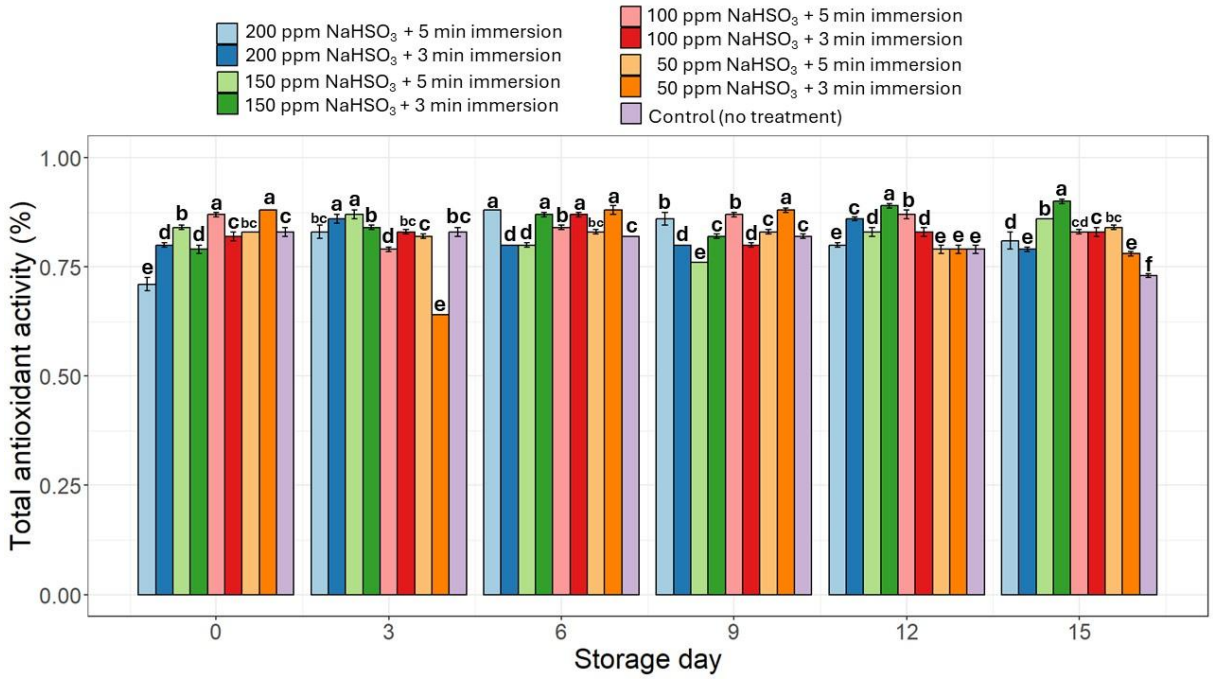


**Fig. 5.** Total antioxidant activity of sliced apples immersed in different sodium bisulfite concentrations and duration after 15 days of storage. The values with the same alphabetic letters and orders were not significantly different according to Duncan's Multiple Range Test (DMRT) with a 5% significance level,

Antioxidant activity in all treatments tended to decrease on day 9. Kunarto and Sani [24] stated that high phenolic and flavonoid content also has high DPPH radical scavenging antioxidant activity. Thus, the decline in antioxidant activity on day nine can also be associated with decreased phenol levels in the same sample (Figure 5). Ref. [25] stated that there is a relationship between phenolic content and antioxidant activity in apples. The decrease in antioxidant activity is thought to be caused by the glycosylation process in the sample during storage. Flavonoid compounds contained in fruit extracts, which are in an impure state, meaning they are bound to other groups, are one of the factors that cause a decrease in antioxidant activity. One of these compounds is a glycoside.

## 4. CONCLUSION

Based on the results and discussion of this research, it can be concluded that soaking fresh-cut Manalagi apples in various concentrations of sodium bisulfite can inhibit changes in the color of the fruit flesh due to enzymatic browning reactions. Then, soaking fresh-cut Manalagi apple fruit in sodium bisulfite with a concentration of 50 ppm for 3 minutes is the treatment with the best ability to inhibit changes in the color of the fruit flesh due to the enzymatic browning reaction.

## ACKNOWLEDGMENT

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