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Efficacy of coconut liquid endosperm as natural agent inhibiting browning and maintaining quality of fresh-cut mature mangoes by comparison with cysteine

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Abstract

The use of natural substances to preserve the freshness of fresh-cut products has been receiving a lot of attention recently. The aim of the present work was to determine the efficacy of coconut liquid endosperm (CLE) immersion on browning retardation and quality maintenance of fresh-cut mature green mangoes by using 'Kaew Kamin' mango as fresh-cut fruit model compared with cysteine (Cys) immersion during refrigeration at 4°C. In the preliminary investigation, the treatment of CLE or Cys retarded the colour change (Δ hue angle) and browning development during refrigeration. The optimal concentrations of CLE and Cys were 100 and 1.5%, respectively. Compared with untreated samples (100% CLE), 1.5% Cys and Cys + CLE immersions maintained firmness, superficial colour, and visual appearance, as well as inhibited the browning and polyphenol oxidase (PPO) activity of the fresh-cut mangoes. Interestingly, Cys immersion enhanced antioxidant activity and the total phenolic compounds of the fresh-cut mangoes. The total phenolic content of the fresh-cut mangoes was unaffected by CLE immersion. Compared to Cys, CLE is a natural substance that prevents browning, and maintains the freshness of fresh-cut mature green mangoes throughout refrigeration.

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Introduction

The consumption of mature-green mangoes is widespread in south and southeast Asia. Green mangoes are consumed fresh, or used as an ingredient in food preparation. Green mangoes are predominantly made up of starch, which may be turned into sugar once ripened. The presence of numerous organic acids is primarily responsible for the sour taste of green mangoes (Pino *et al*., 2005). Browning development after cut is recognised as a main factor affecting overall quality of fresh-cut green mangoes (Naeem *et al*., 2018). The process of wounding intact fruit tissue in order to obtain fresh-cut fruit encourages the development of undesirable colour changes due to enzymatic browning (Nongtaodum and Jangchud, 2009; Marín *et al*., 2021). As a result, the shelf life of fresh-cut fruit is significantly less than that of whole fruit. In this context, the fresh-cut mango sector must achieve a compromise between adequate shelf life and attractive organoleptic properties in order to deliver a superior product that fulfils the expectations of all stakeholders. The delay of flesh browning is therefore an important component in preserving their attractiveness.

Antibrowning chemicals such as cysteine and synthetic ascorbic acid are commonly used in the fresh-cut industry (Ali *et al*., 2016a; 2016b; Marín *et al*., 2021). They are highly effective inhibitors of polyphenol oxidase (PPO). Cysteine has been researched as one of the most effective antibrowning agents on several fresh cut fruits (Gorny *et al*., 2002; Guerrero-Beltran *et al*., 2005; Bico *et al*., 2009; Colantuono *et al*., 2015). Furthermore, cysteine has been certified as Generally Recognized as Safe (GRAS); accordingly, its usage in the worldwide food business would be regarded safe, with no risks to human health. Cysteine typically binds to *o*-quinones, forming coloured compounds known as 'cysteinyl-adducts,' which have been found to be competitive inhibitors of PPO (Richard-Forget *et al*., 1992). Wen

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et al. (2020) opined that low-dose cysteine is a powerful PPO competitive inhibitor. According to Ali *et al*. (2016b), high concentration of cysteine immediately interacts with quinone to produce colourless adducts. However, cysteine generates an undesirable odour, according to Queiroz *et al*. (2008). Wen *et al*. (2020) reported that the use of ultrasonic combined with low-concentration cysteine inhibited browning incidence in lotus root slices without imparting an undesirable flavour.

The use of chemical compounds as antibrowning agents is prohibited under food safety regulations. Meanwhile, most people's apprehension about utilising chemical compounds in foods has increased over time. As a result, applying natural agents in order to satisfy consumers' demands might be a better approach. Many natural agents and/or plant extracts, such as pineapple juice (Lozano-de-Gonzalez *et al*., 1993), rhubarb juice (Son *et al*., 2000), essential oil from plants (Weerawardana *et al*., 2020), and tannins from durian shells (Liang *et al*., 2022) have been shown to control browning in fresh-cut fruits and vegetables. Compounds discovered in those natural agents have confirmed the hypothesis of their ability to inhibit PPO activities. Waste from the coconut milk and copra industries is mature coconut liquid endosperm. Coconut liquid endosperm has been shown to be an antibrowning agent for fresh-cut apples, inhibiting oxidative processes including enzymatic browning (Supapvanich *et al*., 2018).

The purpose of the present work was therefore to assess the efficacy of coconut liquid endosperm and cysteine on browning incidence and qualitative aspects of fresh-cut green mango. Fresh-cut green 'Kaew Kamin' mango was used as model since it is a commercial cultivar consumed in its green stage. For that purpose, the influence of individual coconut liquid endosperm (CLE) or cysteine solutions and combined solutions on the physicochemical characteristics of fresh-cut mature mango was determined.

Materials and methods

Raw material preparation and treatments

Mangoes (*Mangifera indica* L.) cv. 'Kaew Kamin' were harvested at the stage of 90 d (60% maturity) after anthesis. The green mangoes were delivered from a mango orchard to laboratory within 30 min after harvest. The experiment was conducted immediately when the fruits arrived. The fruits were cleaned with water, and soaked in 200 µL L^{-1} sodium hypochlorite for 2 min. After that, the fruits were peeled, and three pieces were longitudinally cut per half fruit. In the preliminary investigation, the fresh-cut green mangoes were immersed in CLE at concentrations of 25, 50, 75, and 100% (v/v) or Cys at concentrations of 0.5, 1.0, and 1.5% (w/v) for 2 min. Untreated fresh-cut mango was used as control. The changes in colour (Δ hue angle) and browning index (BI) were monitored during refrigeration at 4°C for 6 d. To evaluate the effectiveness of CLE on browning inhibition and firmness maintenance to Cys, which was employed as a reference antibrowning agent, the best treatment of CLE preventing browning incidence in the fresh-cut green mangoes was chosen. The fresh-cut green mangoes were immersed in CLE, Cys, or Cys mixed with CLE solution (Cys + CLE) for 2 min. Untreated fresh-cut mango was used as control. In all experiments, the fresh-cut green mangoes were packed in a polyethylene terephthalate clamshell container (~200 g per container), and then stored at 4°C for 6 d. Five replicates (five packages) of each treatment were sampled every 2 d. The changes in texture, visual appearance, superficial colour (*L**, hue, and chroma) values, BI value, PPO activity, total phenolic content, and antioxidant activity were monitored during the refrigeration.

Appearance and colour measurements

Photos of the fresh-cut mature green mangoes obtained during refrigeration for 0 and 6 d were used to assess how the appearance changed. The cut-surface colour of the fresh-cut mangoes was measured using a Minolta chromameter, DP-300 (Minolta, Japan). The *L**, *a**, *b**, hue, and chroma values of fresh-cut green mangoes were recorded. BI was calculated using the formula of Palou *et al*. (1999). The Δ hue value was determined and reported as colour difference compared with the colour on the initial day, as shown below (Eq. 1):

$$\Delta \text{ hue} = \sqrt{\left(hue\ (initial\ day) - hue\ (sampling\ date)\right)^2} \quad \text{(Eq. 1)}$$

Texture measurement

The texture of the fresh-cut mangoes was determined using a TA Plus Texture Analyser (LLOYD Instruments, TA plus Ametek, UK). A 3 mm diameter cylinder probe was used to measure the

firmness. The probe was pushed to a depth of 5 mm at a crosshead speed of 5 mm s^{-1}. The highest force required to compress the middle part of a fresh-cut mature green mango piece was recorded as Newtons (N).

Polyphenol oxidase activity assay

A 5 g of the cut-surface of fresh-cut mature green mangoes was homogenised with 15 mL of cold 0.2 M phosphate buffer (pH 7) containing 0.5 g of polyvinylpolypyrrolidone. The PPO activity was assayed using the method of Galeazzi *et al*. (1981) with slight modification by Wen *et al*. (2020). The supernatant was reacted with a 0.05 M catechol solution at pH 6.8. PPO activity unit (U) was specified as increased optical density (OD) at 420 nm by 0.001 min^{-1} at room temperature (29 ± 1°C), and the U per kilogram fresh weight (U kg^{-1}) was reported.

Total phenolic compounds assay

A 5 g of the cut-surface of the samples was extracted with 60% (v/v) of ethanol. The concentration of total phenolic compounds was determined using the method of Slinkard and Singleton (1997) with slight modification by Wen *et al*. (2020). The supernatant was reacted with Folin-Ciocalteu solution and saturated sodium carbonate. A recording of the OD at 750 nm was made. The concentration of total phenolic compounds was computed using an equation derived from a standard curve of gallic acid, and expressed as g gallic acid equivalent per kilogram fresh weight (g kg^{-1}).

Antioxidant activity assay

A 5 g of fresh-cut mature mangoes was extracted using the same extraction method describe earlier. The supernatant was used to determine antioxidant activity using the ferric-reducing antioxidant potential (FRAP) assay. The method was described by Benzie and Strain (1996) with slight modification by Wen *et al*. (2020). The extract was reacted with FRAP reagent, and a recording of the OD at 630 nm was made. The antioxidant capacity was expressed as mol Trolox equivalent per kilogram fresh weight (mol kg^{-1}).

Statistical analysis

Completely randomised design (CRD) was used to design the experiments. The data were presented as mean and standard deviation of five replications. The data variance was determined using the analysis of variant (ANOVA), and the means comparison were analysed using Duncan's Multiple Range test (DMRT) at the significant level of 0.05.

Results and discussion

Preliminary study

The efficacy of CLE or Cys immersion at various concentrations on the Δ hue angle value and BI development of fresh-cut mature green mangoes during refrigeration were preliminarily observed. All CLE or Cys treatments delayed the increase in Δ hue angle value and BI of fresh-cut mature mangoes during refrigeration (Figure 1). The concentration of CLE and Cys was favourably linked with the effectiveness of CLE and Cys immersions in retaining colour and preventing browning development. The Δ hue angle and BI of untreated fresh-cut mature mangoes were significantly higher than those of the fresh-cut mature mangoes treated with CLE and Cys throughout refrigeration ($p < 0.05$). The treatment with 100% CLE delayed the increase in Δ hue angle and BI value, making it better than other concentrations. On day 6 of storage, 100% CLE treated fresh-cut mature mangoes had significantly lower Δ hue angle and BI values than other CLE treated samples ($p < 0.05$). This proved that CLE inhibited fresh-cut mature mangoes from turning brown or discolouration during refrigeration. Supapvanich *et al*. (2018; 2020) also reported that the immersions of liquid endosperm from both young and mature coconuts inhibited browning development and maintained the whiteness of fresh cut 'Gala' apples during storage due to the retardation of browning enzyme activity. A recent study showed that 1.5% Cys immersion significantly inhibited the increase in Δ hue angle and BI values compared to other concentrations ($p < 0.01$). Cys is accepted as an effective antibrowning agent that has been applied to fresh-cut fruit and vegetables (Pace *et al*., 2015; Ali *et al*., 2016b; Wen *et al*., 2020). The application of Cys at 1.5% to maintain visual appearance and inhibit browning development of fresh cut 'Nam Dok Mail' mangoes during cold storage was reported by Techavuthiporn and Boonyaritthongchai (2016). Regarding the results shown in Figures 1(Ba) and 1(Bb), 100% CLE and 1.5% Cys immersion were chosen for subsequent experiments on the texture and factors affecting the development of browning in fresh-cut green mangoes.

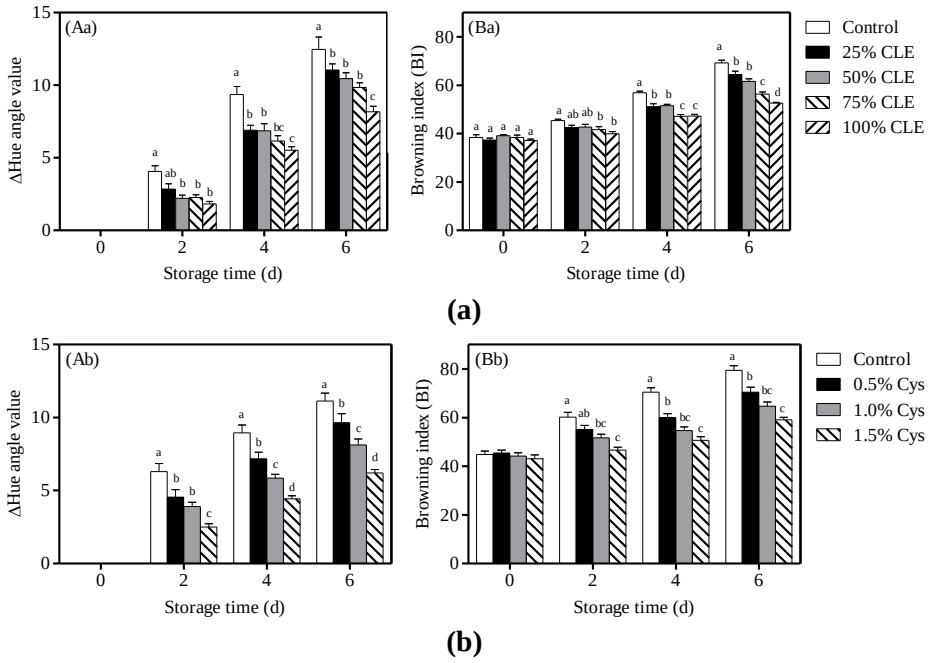

Figure 1. Hue angle value **(A)** and browning index **(B)** of CLE- **(a)** and Cys- **(b)** treated fresh-cut mangoes during refrigeration at 4°C for 6 d. On each sampling date, different lowercase letters indicate significant difference between treatments at $p < 0.05$.

Texture

Figure 2 shows the change in firmness of fresh-cut mature mangoes treated with CLE, Cys, and Cys + CLE during refrigeration. Compared to untreated samples, the firmness of all treated fresh-cut mature mangoes was preserved. The firmness of control samples decreased throughout storage, and was significantly lower than that of the treated fresh-cut mature green mangoes ($p < 0.05$). However, the firmness of CLE, Cys, and Cys + CLE treated fresh-cut mature mangoes was not different during refrigeration. Compared to the hardness on the first day of storage, fresh-cut mature green mangoes treated with CLE, Cys, and Cys + CLE showed decrease in firmness of 18.99, 10.49, and 8.56%, respectively, at the end of storage (day 6). This indicated that the firmness of fresh-cut mature mangoes was preserved by CLE, Cys, or Cys + CLE immersion. Gohari *et al*. (2021) reported that the application of L-cysteine delayed the softening of peach fruits, which might be related to the decreased activities of cell wall hydrolases such as pectin esterase and polygalacturonase. Moreover, previous works also reported that L-cysteine delayed the increased fruit softening due to the retardation of membrane dysfunction and cell wall degradation (Li *et al*., 2018). Wen *et al*. (2020) also reported that fresh-cut lotus root treated with L-cysteine had higher firmness than the untreated samples. The impact of coconut water on preserving the firmness of fresh-cut fruits has not yet been documented. Kwiatkowski *et al*. (2008) reported that K and Ca are major mineral elements in coconut water. Ca plays an important role in maintaining the firmness of fruits and vegetables due to calcium pectate formation. Therefore, the Ca content in CLE might play a role in preserving the firmness of fresh-cut mature mangoes during storage. Moreover, we found that the firmness of CLE treated fresh-cut mature mangoes was not different from that of Cys treated fresh-cut mature mangoes during storage.

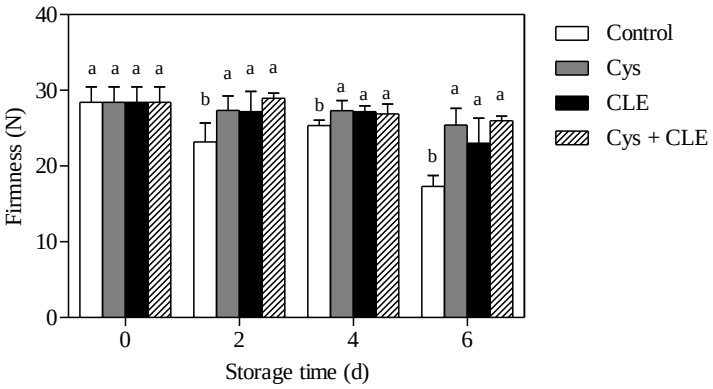

Figure 2. Firmness of fresh-cut mangoes treated with Cys, CLE, and Cys + CLE during refrigeration at 4°C for 6 d. On each sampling date, different lowercase letters indicate significant difference between treatments at $p < 0.05$.

Superficial colour and appearance

The changes in colour attributes and visual appearance of fresh-cut mature mangoes during refrigeration are presented in Figure 3. There were no significant differences in *L**, hue angle, or BI values of each treatment during storage for 4 d. All treatments had no direct effect on the change in chroma value over storage. After storage for 6 d, the *L** and hue angle values of untreated fresh-cut mature mangoes were significantly lower than those of Cys, CLE, and Cys + CLE treated fresh-cut mature mangoes ($p < 0.05$). CLE, Cys, and Cys + CLE immersions significantly inhibited the increase in BI compared to control ($p < 0.05$). The changes in superficial colour attributes were correlated with the appearance of fresh-cut mature mangoes, in which browning occurrence was found in control after storage for 6 d, whereas no browning incidence was noticed in Cys, CLE, or Cys + CLE treated fresh-cut mature mangoes. The sudden increase in BI value in control might be associated with the onset of the deterioration process after storage for a range of times. These indicated that CLE immersion maintained superficial colour and visual appearance as well as retarded browning of fresh-cut mature mangoes, being equal to Cys and Cys + CLE immersions during refrigeration for 6 d. The effectiveness of CLE and Cys on browning development inhibition was described by Pace *et al.* (2015), Ali *et al.* (2016b), and Supapvanich *et al.*

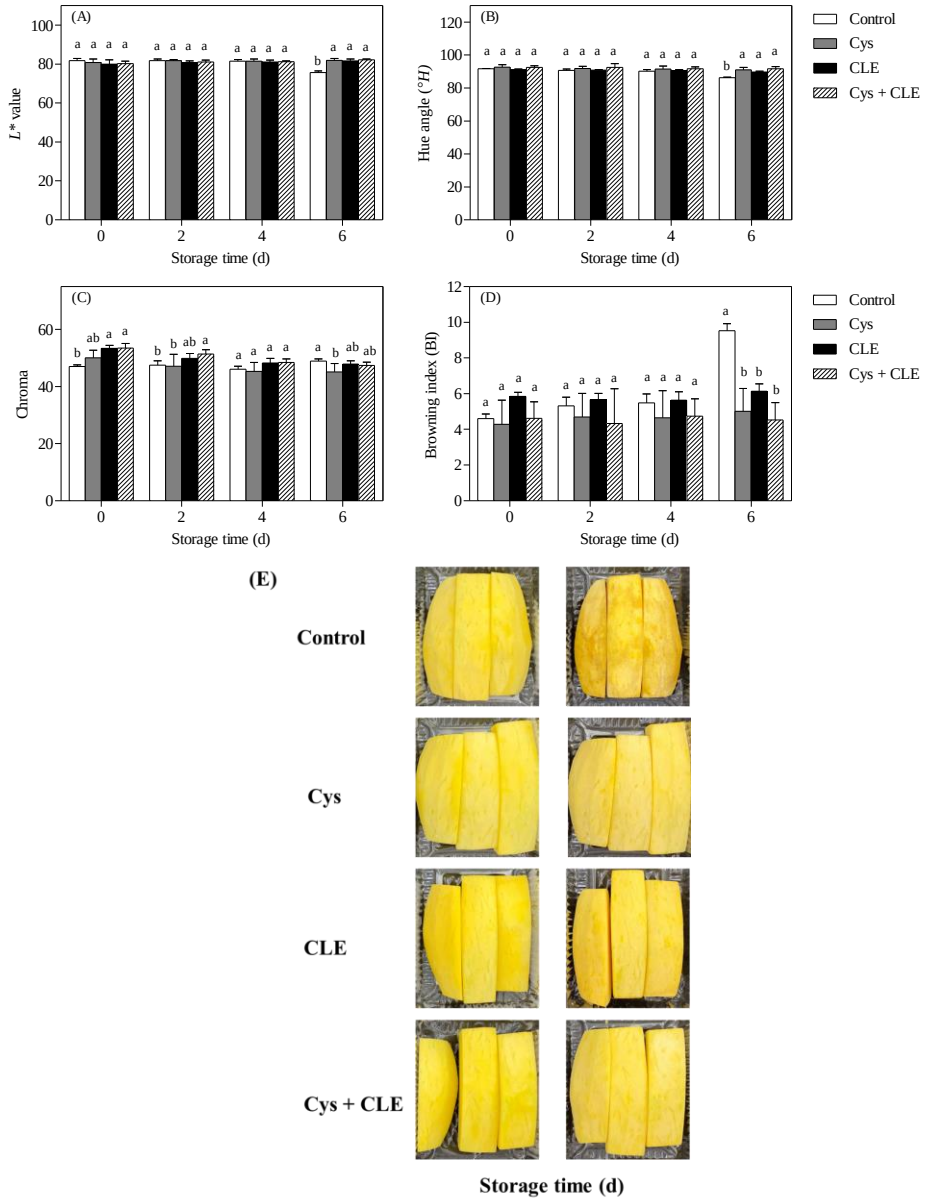

Figure 3. *L** **(A)**, hue angle **(B)**, chroma **(C)**, browning index **(D)**, and visual appearance **(E)** of fresh-cut mangoes treated with Cys, CLE, and Cys + CLE during refrigeration at 4°C for 6 d. On each sampling date, different lowercase letters indicate significant difference between treatments at $p < 0.05$.

(2020). Moreover, we found that Cys + CLE immersion could retard the browning occurrence of fresh-cut mature mangoes, and it was not different from the immersion of CLE or Cys alone. It was clearly demonstrated that enzymatic browning on the cut-surface of fresh-cut mature green mangoes was prevented by both CLE and Cys immersions.

Polyphenol oxidase activity and total phenolic content

Increases in PPO activity and total phenolic content are signs that fruit and vegetable enzymatic browning is occurring (Wessels *et al*., 2014). The PPO activity and total phenolic contents of the cut-surface of fresh-cut mature green mangoes are shown in Figure 4. An increase in PPO activity in all samples was detected during refrigeration (Figure 4A). The increase in PPO activity in untreated fresh-cut mature mangoes was higher than that of Cys, CLE, and Cys + CLE treated samples. No significant difference in PPO activity between Cys, CLE, and Cys + CLE treated fresh-cut mangoes was observed over refrigeration for 6 d. On day 6, the PPO activity of untreated fresh-cut mature green mangoes was significantly higher than that of other treated samples ($p < 0.05$). The total phenolic content of Cys treated fresh-cut mature green mangoes was significantly higher than that of control and CLE treated samples during refrigeration ($p < 0.01$) (Figure 4B). However, no significant difference in total phenolic content between Cys and Cys + CLE treated fresh-cut mature green mangoes was observed during storage. The CLE immersion had no effect on the change in total phenolic content of fresh-cut mature green mangoes compared to untreated samples. This showed that Cys might increase the amount of total phenols in fresh-cut mature green mangoes. Preczenhak *et al*. (2019) suggested that Cys enhanced total phenolic compounds in fresh-cut beet roots due to the induction of phenylalanine ammonia lyase (PAL) activity. Gohari *et al*. (2021) also reported that Cys treatment induced PAL activity, and reduced PPO activity, which led to an increment in total phenolic content, and the retardation of browning development in peach fruits. Additionally, the study discovered that Cys immersion in fresh-cut mature green mangoes increased the quantity of total phenolic compounds, and inhibited the increase in PPO activity. These indicated that Cys could induce phenolic compound biosynthesis, and inhibit the enzymatic browning reaction. In the case of CLE immersion, the inhibition of PPO activity of fresh-cut mature mangoes might be associated with the high antioxidant capacity of CLE as described by Supapvanich *et al*. (2020). Moreover, CLE has high content of salicylic acid, which is an effective competitive inhibitor of PPO (Zhou *et al*., 2015). Our previous works also reported that the change in total phenolic content was not related to browning development in fresh-cut apples treated with CLE (Supapvanich *et al*., 2018; 2020).

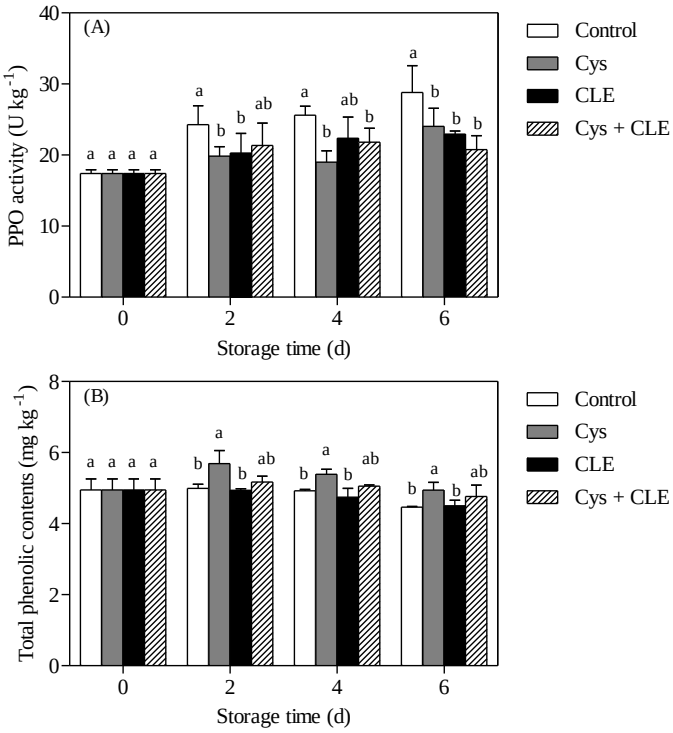

Figure 4. Polyphenol oxidase **(A)** and total phenolic content **(B)** of fresh-cut mangoes treated with Cys, CLE, and Cys + CLE during refrigeration at 4°C for 6 d. On each sampling date, different lowercase letters indicate significant difference between treatments at $p < 0.05$.

Antioxidant activity of fresh-cut mature mangoes treated with Cys, CLE, and Cys + CLE

The change in antioxidant capacity of the fresh-cut mangoes is shown in Figure 5. The increased antioxidant activity in fresh-cut products retarded oxidative reactions, including enzymatic browning reaction. Like total phenolic content, Cys and Cys + CLE enhanced the antioxidant capacity of fresh-cut mature green mangoes during refrigeration. The antioxidant capacity of Cys and Cys + CLE treated samples was significantly higher than that of

control and CLE treated samples throughout storage. No significant difference in the antioxidant activity of Cys and Cys + CLE treated fresh-cut mature mangoes was detected throughout refrigeration. The antioxidant capacity of control and CLE treated fresh-cut mature mangoes was similar during storage for 4 d. After storage for 6 d, the antioxidant capacity of CLE treated fresh-cut mature mangoes was significantly higher than that of control ($p < 0.05$). This indicated that Cys could induce antioxidant capacity in the fresh-cut mature mangoes, whilst CLE might have no effect on the antioxidant capacity enhancement. The antioxidant enhancement by Cys is due to the induction of PAL activity, and the stimulation of phenolic compound biosynthesis, as described by Preczenhak *et al*. (2019) and Gohari *et al*. (2021). The high antioxidant capacity of CLE treated fresh-cut mature mangoes compared to untreated samples might be associated with CLE absorption during immersion.

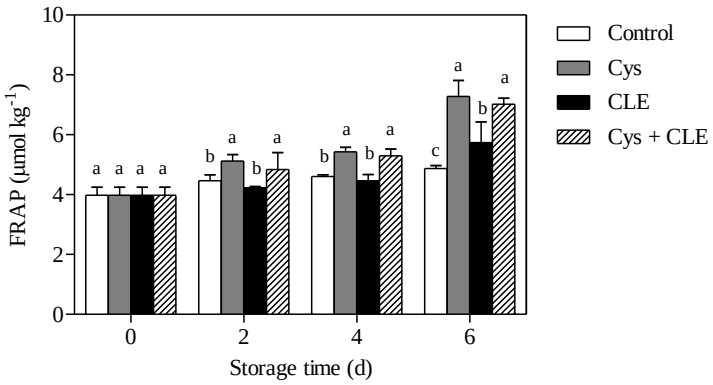

Figure 5. Ferric-reducing antioxidant potential of fresh-cut mangoes treated with Cys, CLE, and Cys + CLE during refrigeration at 4°C for 6 d. On each sampling date, different lowercase letters indicate significant difference between treatments at $p < 0.05$.

Conclusion

Immersion in Cys or CLE retained colour and reduced browning incidence in fresh-cut mature green mangoes throughout 6-d storage at 4°C. The 1.5% Cys and 100% CLE maintained the colour and prevented the browning increase more than other concentrations. Compared with untreated fresh-cut mangoes, the Cys, CLE, and Cys + CLE immersions clearly preserved firmness, visual appearance, and superficial colour characteristics while also inhibiting browning during refrigeration. The PPO activity of the fresh-cut mango cut surface was also inhibited by Cys, CLE, or Cys + CLE immersion. Cys treatments induced the total phenolic content of the fresh-cut mangoes. However, the change in total phenolic content had no influence on increased browning during storage. The antioxidant capacity of the fresh-cut mangoes was enhanced by both Cys and Cys + CLE treatments more than by CLE alone. These indicated that CLE immersion could be an alternative natural agent preventing browning and maintaining the firmness of fresh-cut mature green mangoes, and its effect was not different from Cys or Cys + CLE immersion.

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