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Lotus flower extract as a natural anti-browning agent for fresh romaine lettuce (*Lactuca sativa* L. var. *longifolia*)

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Abstract The effect of lotus (*Nelumbo nucifera* Gaertn.), butterfly pea (*Clitoria ternatea)* and Siam tulip (*Curcuma sessilis)* aqueous extract on reduction of polyphenol oxidase (PPO) and peroxidase (POD) in romaine lettuce (*Lactuca sativa* L. var. *longifolia*) were evaluated. Results showed that *in vitro* lotus flower aqueous extract exerted the highest reduction of PPO and POD activities on the romaine lettuce. The effectiveness of *in vivo* application of lotus flower aqueous extract to control browning cut stem ends of fresh romaine was examined. Romaine lettuce harvested as a whole plant and cut off at the stem. Cut stem ends of romaine lettuce were dipped in various concentrations of lotus flower extract for 5 min and packaged in polypropylene plastic bags. Weight loss, color, browning index as well as PPO and POD activities were evaluated during 12 days of storage at temperature of 10±2°C and relative humidity 50±5%. Cut stem ends dipped in 0.5% aqueous extract of lotus flower resulted in inhibition of browning of cut stem ends and decrease in PPO and POD activities. Thus, exogenous 0.5% aqueous lotus flower extract treatment could be a useful application to alleviate browning in cut stem ends of fresh romaine lettuce.

Keywords: Browning, Colour, Polyphenol oxidase, Peroxidase, Weight loss

Introduction

The herbaceous plant known as romaine lettuce (*Lactuca sativa* L. var. *longifolia*), is indigenous to Asia and the Mediterranean region of Europe. When lettuce stems were cut, wounded cells experienced an increase in metabolism and oxidative reactions, which led to stem browning and other metabolic changes (Pace *et al*., 2015). According to Tomas-Barberan and Espin (2001), enzymatic browning of fresh cut produce appears to be caused by oxidation by polyphenol oxidase (PPO) and peroxidase (POD), as well as for whole romaine lettuce that turns brown on the stem end cut-surface. It is common knowledge that browning, which results in a dark color in fresh cuts, is caused by melanin accumulation and formation, which begins with the oxidation of phenols to quinones and end with

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melanin by PPO and POD in the presence of oxygen (Hunt *et al.*, 1993; Jiang *et al*., 2016; Zhu *et al*., 2022). PPO activity was suppressed by 0.5 M KCl, 0.5 M NaCl, and 0.5 M NaBr to regulate lettuce browning (Hisaminoto *et al*., 2001). PPO activity was inhibited by 90% with 5 mmol ascorbic acid, and POD did not to be involved in browning of lettuce (Landi *et al*., 2013).

The effect of employing natural extracts to suppress the browning reaction has been explored, and natural preservation agents that limit browning while also promoting health have gained favor in recent years. Natural compounds, such as eugenol, have been shown to suppress the PAL, PPO, and POD enzyme activity in lettuce (Chen *et al*., 2017). Grape seed extract can inhibit PPO and protect phenolic compounds (Altunkaya and Gökmen, 2011). Heated grape leaf extract significantly prevened lettuce browning (Altunkaya, 2012). Vegetable flowers, fruit flowers, or fragrant flowers have all been consumed as edible flowers for a very long time. Some edible flowers contain major biologically active components like phenolics, flavonoids, and anthocyanin; these components have antioxidant properties and can prevent enzymatic browning of fruit and vegetables (Zheng *et al*., 2019; Śmiechowska *et al*., 2021; Jadhav *et al*., 2023).

In this work, the effects of three edible flowers, including the lotus (*Nelumbo nucifera* Gaertn.), butterfly pea (*Clitoria ternatea)* and Siam tulip (*Curcuma sessilis)*, on PPO and POD activities in romaine lettuce were examined. Then, the effectiveness of *in vivo* application of flower aqueous extract with high PPO and POD reduction to control browning cut stem ends of fresh romaine lettuce was examined.

Materials and methods

Plant materials

Romaine lettuce was used as a raw material for PPO and POD sources and tested plant. The edible flowers of lotus, butterfly pea and Siam tulip were used as raw materials for obtaining aqueous extract.

Edible flowers extract

The fresh edible flowers of lotus were harvested in ponds in the morning at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. Butterfly pea and Siam tulip were purchased from local commercial growers in Bangkok and Nakhon Nayok province, respectively. After three days of hot air oven drying at 45°C, the flower samples were ground to a fine powder (100 mesh) with an electric blender. The dried flower power (25 g) was extracted by soaking

in 0.5 L of water at 4°C for 72 h. Before filtering through filter paper (Whatman No. 1), the obtained aqueous extracts were filtered through four layers of cheesecloth to remove fiber debris to yield solutions containing soluble extracts from 5 g dry plant tissue per 100 mL. The extracts were kept in sealed containers at 4°C until further use.

Edible flower extract on PPO and POD activity reduction

For source of PPO and POD enzymes, 45 grams of romaine lettuce were cleaned and washed. The romaine lettuce was chopped and then added 150 mL of in 0.05 M phosohate buffer (pH 7.0) with 25 g/L polyvinylpolypyrrolidone (PVPP) during homogenation in an electric commercial blender. All steps were carried out at 4-8°C. The slurry was filtered through two layers of cheesecloth, followed by a filter paper filter. The supernatant served as a crude enzyme source for PPO and POD assays, after filtration. The Bradford (1976) method was employed to determine the protein content, with bovine serum albumin as the standard. The spectrophotometric determination of PPO activity was conducted using a visible spectrometer at a wavelength of 398 nm, employing catechol as the substrate (Chen *et al*., 2017). The mixture was prepared by combining 3 mL of a 0.5 M catechol solution in a 0.1 M phosphate buffer (pH 7.0), 0.1 mL of a treatment solution containing 1% flower extract, 0.17% ascorbic acid, and water, and finally, 0.2 mL of a crude enzyme extract. The activity of POD was assessed using a visible spectrometer at a wavelength of 470 nm, employing guaiacol as the substrate and H_2O_2 as the hydrogen donor, as described by Mousavizadeh and Sedaghathoor (2011). The mixture consisted of 3 ml of 0.4% guaiacol, 0.1 mL of 0.46% hydrogen peroxide, 2 mL of 0.05 M phosphate buffer, 0.1 mL treatment solution (1% edible flower extract, 0.17% ascorbic acid and distilled water) and 0.5 mL crude enzyme extract. One unit of enzyme activity was defined as the amount of enzyme required to produce a 0.01 absorbance increase per minute under assay conditions. The enzyme activity was expressed as U/mg protein.

Application of lotus aqueous extract in fresh romaine lettuce

Romaine lettuce harvested as a whole plant was obtained from a local grower in Bangkok, Thailand and immediately transported to the laboratory within 2 hours. Romaine lettuce was washed and selected a uniform size and without signs of diseases and pets and then cut on the stem using a sharp knife. Their cut ends were dipped in lotus extract in concentrations of 0, 0.05, 0.1 and 0.5% for 5 minutes, air-dried and packaged in polypropylene plastic bags. The treated samples were stored at 10±2°C for 12 days. Romaine lettuce of each

treatment at storage time of 0, 3, 6, 9 and 12 days were analysed to evaluate the weight loss, color change, browning score, browning index as well as PPO and POD activities.

Weight loss

Fresh weights of cut flowers were measured every three days. The following formula was used to determine relative weight:

$$\text{Weight loss (\%)} = \frac{W_0 - W_t}{W_0} \times 100$$

where W_t represents the fresh weight on the day of observation and W_0 represents the initial fresh weight.

Colour measurement

The surface colour of the cut stem ends of romaine lettuce was examined using a colorimeter (Konica Minolta, CR-10 Plus) with a measurement area 8 mm round. Parameters L*(lignt/dark), a* (red/green) and b*(yellow/blue) were measured.

Determination of browning

The browning index (BI), defined as brown colour purity was determined according to the following formula:

$$BI = \frac{100\,(x - 0.31)}{0.17}$$

Where $x = \frac{(a^* + 1.75L^*)}{5.645L^* + a^* - 3.012b^*}$.

Appearance score evaluation

Browning of the cut surfaces was scored on stem parts of fresh romaine lettuce using a four-point scale, where 1 = severe browning, 2 = moderate browning, 3 = slightly browning and 4 = no browning.

According to Chinwang *et al.* (2011), the visual freshness score of romaine lettuce was assessed using a scale ranging from 4 to 1, scoring 4 (excellent), 3 (good), 2 (average) to 1 (poor).

PPO and POD activities of cut stem ends of romaine lettuce during storage

For analysis of enzymatic activities, stem ends of romaine lettuce (10 g) were homogenized with 30 mL of in 0.05 M phosohate buffer (pH 7.0) with 25 g/L PVPP. After filtration, the supernatant served as a crude enzyme source for

PPO and POD assays. PPO and POD activitis was assessed spectrephotometrically according to Chen *et al*. (2017) and Mousavizadeh and Sedaghathoor (2011), respectively.

Statistical analysis

Experiments were performed using a completely randomised design. The data were subjected to one-way analysis of variance using the SPSS statistical software, and differences among treatments were evaluated by Tukeys test at $p \leq 0.05$.

Results

Screening of PPO and POD activity reduction by edible flower extract

The inhibitory effects of the aqueous extract derived from Siam tulip, butterfly pea, and lotus flowers on enzymes associated with the browning process *in vitro* are illustrated in Figure 1A. The control samples without flower extract exhibited the highest level of PPO activity, while the lowest level of PPO activity was detected in the ascorbic acid (standard). The activity of PPO was found to decrease in the presence of 1% butterfly pea extract (62539.13 units/mg protein) and 1% lotus extract (62075.01 units/mg protein) as compared to the control group (69825.69 units/mg protein). The results of the experiment demonstrated that the application of edible flower extract on romaine lettuce extract led to a notable decrease in POD activity. Specifically, the Siam tulip and lotus extracts exhibited POD activity reduction of 32320.78 and 32627.09 units/mg protein, respectively, in comparison to the control group which had a POD activity of 33945.17 units/mg protein (Figure 1B).

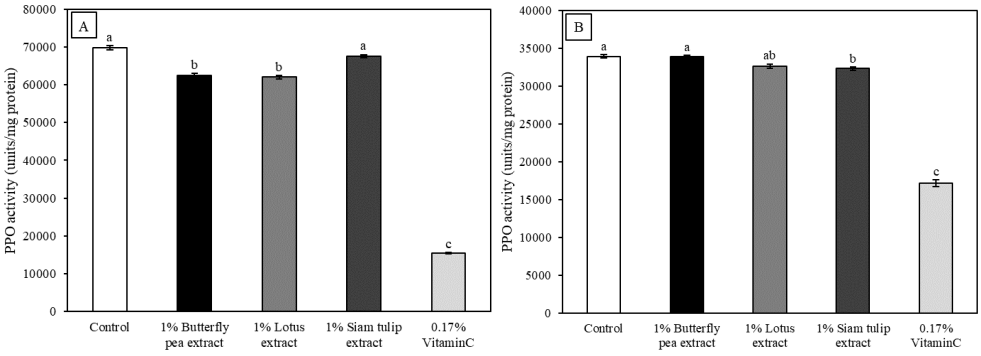


Figure 1. Effect of flower aqueous extract and ascorbic acid on PPO (A) and POD (B) activities in romaine lettuce

Application of lotus extract in fresh romaine lettuce

The aqueous extract of the lotus flower exhibited the most inhibitory effect on the PPO and POD activities *in vitro* (Figure 1). Based on the obtained findings, there is a need for additional investigation into the efficacy of lotus aqueous extract as an anti-browning agent on alleviating browning on cut-surface of fresh romaine lettuce. Weight loss, color, browning index, and PPO and POD activity were assessed over the course of 12 days of storage after the cut ends of romaine lettuce were immersed in 0.01, 0.1, and 0.5% lotus flower extract for 5 min.

Weight loss

The weight loss of romaine lettuce increased with the duration of storage for all treatments, as shown in Figure 2. The weight loss was observed in the control samples, 0.05, 0.1 and 0.5% lotus extract, they reached 13.05, 12.24, 13.78 and 11.01% at the end of storage, respectively. However, no significant differences were observed between flower extract treatment and control samples throughout storage time.

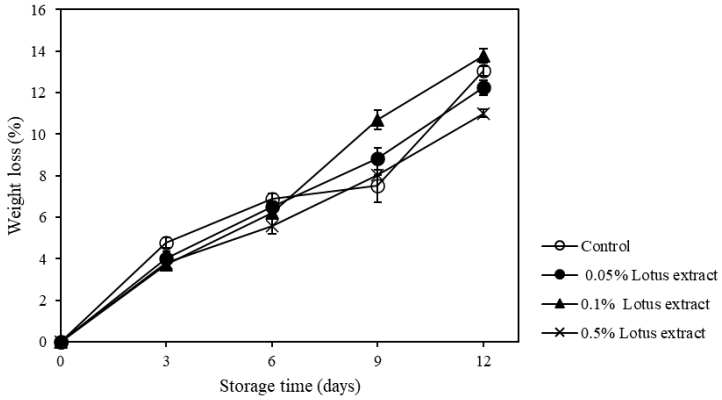


Figure 2. Effect of lotus extract treatments on weight loss of fresh romaine lettuce during storage at 10±2°C

Colour change (L, a* and b* value)*

Changes in the stem end colour were evaluated by measuring L*(light/dark), a* (red/green) and b* (yellow/blue) according to Figure 2. On day 9 after storage, the L* values of the control samples and those treated with 0.05% lotus extract were found to be significantly lower than samples treated with lotus extract at concentrations of 0.1% and 0.5% (Figure 3A). However, at the end of the storage period, there were no significant variations in L* values among

treatments. Regardless of the treatments, the a* values of the stem cut surface exhibited a shift from negative to positive, suggesting a change from green to red color components (Figure 3B). The b* values of cut ends had a tendency to increase as the storage period advanced in all the treatments (Figure 3C). There were no significant differences between flower extract-treated and control samples.

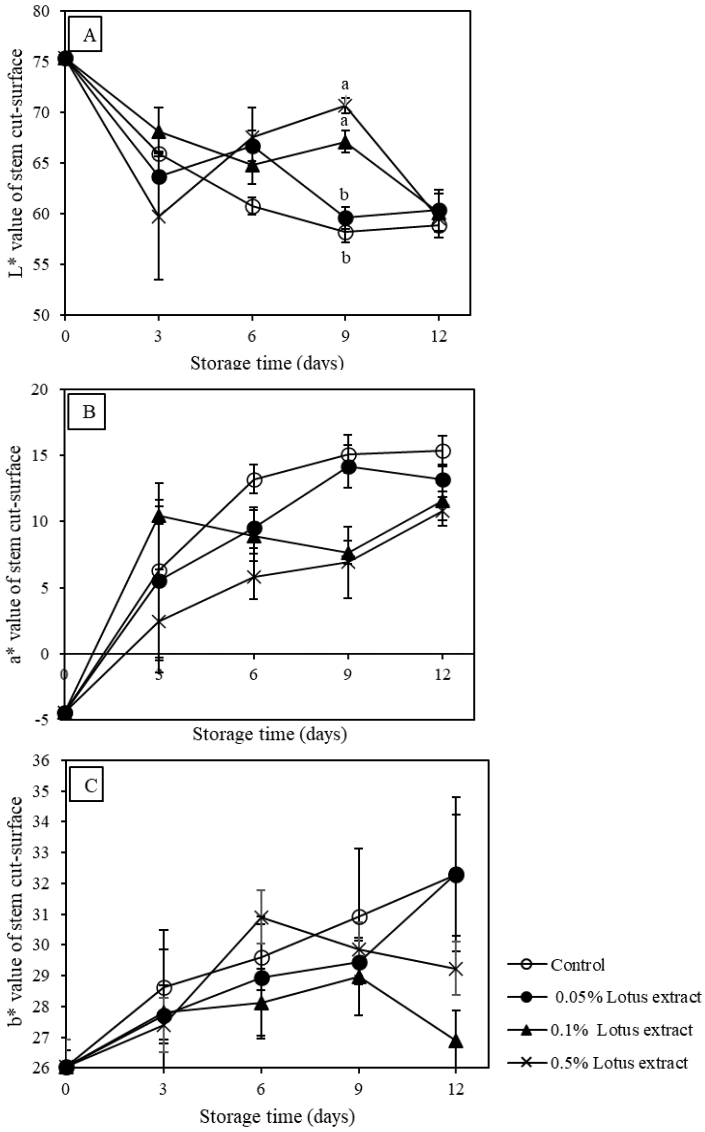


Figure 3. Effect of lotus extract treatments on colour L* values (A), a* values (B), and b* values (C) on stem of fresh romaine lettuce during storage at 10±2°C

Browning index

The initial browning index of stem cut surface of the romaine lettuce was 33.10. The browning index of sample lotus extract treatment at 0.05% was similar to controls but at higher lotus extract concentration exhibited significant lower browing index than the controls on day 9 for 0.1% extract and on day 6 and 9 for 0.5% extract. At the end of storage, the browning index of samples treated with lotus extract at 0.005, 0.1 and 0.5 was 89.67, 72.03 and 78.56, respectively, whereas the control samples was 94.80 (Figure 4).

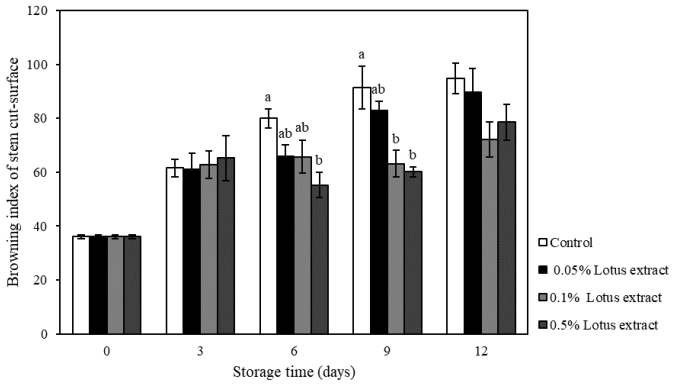


Figure 4. Effect of lotus extract treatments on browning index on cut surface of fresh romaine lettuce during storage at 10±2°C

Browning score

During storage, alterations in browning ratings for the stem cut surface are illustrated in Figure 5A. After 6 and 9 days of storage the cut ends treated with 0.5% lotus extract had a browning score of 3.0 ± 0.2 and 2.3 ± 0.3 significantly higher compared to the scores of control samples of 1.7± 0.3 and 1.0 ± 0.0, respectively. Changes in visual freshness scores for whole samples during storage are described in Figure 5B. Except for after 9 days of storage, there were no statistically significant variations in the visual freshness scores between the treatments during storage. On day 9 romaine lettuce dipped in 0.5% lotus extract (3.0 ± 0.0) had freshness score comparable to 0.1% lotus extract (2.7 ± 0.3) and significant higher freshness score than 0.01% lotus extract (1.7 ± 0.3) and control samples (1.3 ± 0.3), repectively. Higher level of browning and freshness in the samples was scored in romaine lettuce dipped in 0.5% lotus extract. When romaine lettuce was dipped in different concentrations of lotus extract (Figure 6)

and 0.5% lotus extract prevent the browning reaction on its cut surface. It also maintained freshness romaine lettuce (Figure 7).

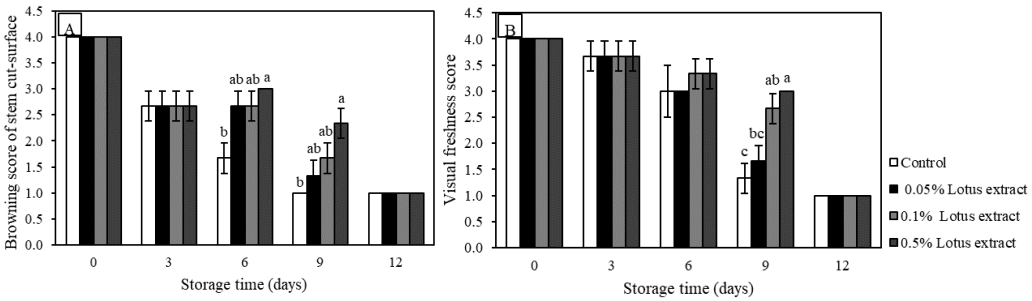


Figure 5. Effect of lotus extract treatments on browning score (A) on stem cut-surface and visual freshness score (B) of whole romaine lettuce during storage at 10±2°C

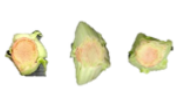
Storage time (days)	Treatment			
	Control	0.05% Lotus extract	0.1% Lotus extract	0.5% Lotus extract
Day 0				
Day 3				
Day 6				
Day 9				
Day 12				

Figure 6. Changes in the photographs of stem cut-suface of romaine lettuce under different lotus extract concentrations of 0, 0.05, 0.1 and 0.5% during storage at 10±2°C for 12 days

Storage time (days)	Treatment			
	Control	0.05% Lotus extract	0.1% Lotus extract	0.5% Lotus extract
Day 0				
Day 3				
Day 6				
Day 9				
Day 12				

Figure 7. Changes in the photographs of whole romaine lettuce under different lotus extract concentrations of 0, 0.05, 0.1 and 0.5% during storage at 10±2°C for 12 days

PPO and POD activity

Changes in the activities of PPO and POD in romaine lettuce after exposed to lotus extract are shown in Figure 8. The initial PPO activity of romaine lettuce was 8330.04 units/mg protein. During the 12-day storage period, PPO activity in the control and treatment samples increased slightly, decreased slightly, and then increased sharply again. On days 6 and 9, PPO activity was significantly higher in untreated samples of stem-cut lettuce than in samples treated with 0.1 and 0.5% lotus extract (Figure 8A).

The initial POD activity of romaine lettuce was 11492.38 units/mg protein. The POD activity of the control samples increased dramatically after three days of storage. On days 6 and 9, the untreated samples increased in POD activity in stem-cut lettuce compared to lotus extract (Figure 8B).

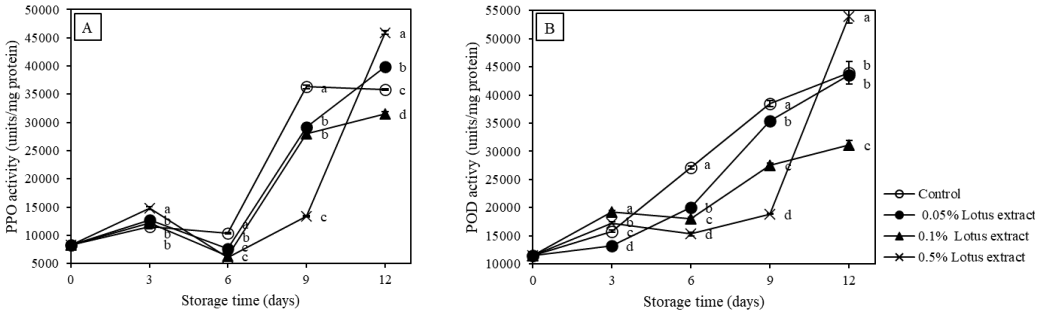


Figure 8. Effect of lotus extract treatments on PPO (A) and POD (B) activity of stem cut-surface of fresh romaine lettuce during storage at 10±2°C

Discussion

The evaluation of vegetable freshness by consumers is influenced by the perceived visual characteristics of vegetables. The primary factors contributing to the reduction in weight of vegetables postharvest are respiration and transpiration (Tano *et al*., 2005). The application of lotus extract by the dipping method did not effectively mitigate weight loss during storage. This observation aligns with the concurrent decrease in the visual freshness score, ultimately leading to a decline in the visual freshness of fresh romaine lettuce. The control samples exhibited a rapid occurrence of browning at the cut surface of the stem, but the romaine lettuce treated with 0.5% lotus extract exhibited a diminished degree of browning. The elucidation of variations in the brown coloration across different treatments can be achieved through the assessment of lightness values, browning index, and browning score. These results suggest that lotus extract delayed the browning on cut surface of fresh romainne lettuce. The degree of browning was evaluated using colorimetry, specifically the lightness (L*) and chromaticity coordinates (a* and b*) of the product. According to Chen *et al*. (2017), there is a correlation between the increase of browning products and a decrease in L* values, as well as an increase in a* and b* values. The lightness values of the cut surface were substantially affected by treatment; 0.5% lotus extract samples had the highest lightness.

The roamaine lettuce samples treated with 0.5% had significantly lower PPO and POD activities, compared with controls. Both enzymes are known as wound-response enzymes that catalyze the oxidation of phenolic substrates into brown compounds, resulting in vegetable discolouration, off-flavors, and nutritional loss (Zhou *et al*., 2015). The lotus plant is widely recognized for its antioxidant properties, which contribute to its significant potential for application

in the food and medicine sectors (Zhu *et al*., 2015; Je and Lee, 2015). According to Wen *et al*., (2020), a variety of flavonol aglycones have been identified in lotus flower, with over 10 such compounds documented in nature. Notably, quercetin, myricetin, kaempferol, and isorhamnetin are among the most often encountered flavonol aglycones. Lotus extract offers a variety of mechanisms of action for preventing enzyme browning since it contains a large number of components. The lotus extract's anti-browning properties likely result from its ability to inhibit PPO or POD directly by converting phenolic molecules to o-quinones before these polymerize into dark melanin pigments. Similarly ascorbic acid, lotus extract also inhibits enzymatic browning by reducing the *o*-quinone compounds and preventing the formation of dark pigments (Oms-Oliu *et al*., 2010). Moreover, quercetin which is one of the compounds found in lotus flowers, exhibits potent antioxidant properties (Singh *et al*., 2021). According to Chen and Kubo (2002), quercetin is a competitive inhibitor of PPO.

This study demonstrated that romaine lettuce weight loss was not slowed down by dipping in lotus extract. The application of 0.5% aqueous lotus flower extract to cut stem ends of fresh romaine lettuce effectively reduced discoloration.

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