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The preservation effects of water chestnut peel extracts on fresh cut apples

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Abstract
This study explored the effects of water chestnut extract on the preservation of fresh-cut apples, which were treated with different concentrations of water chestnut extracts (0.1%, 0.2% and 0.3%). Treatment with water chestnut extracts retarded the decreases in titratable acid and soluble solid contents; inhibited the increase in browning enzyme activity; and effectively maintained the hardness, colour and other storage qualities of fresh-cut apples. On the 10th day of storage, the contents of titratable acid and soluble solids in fresh-cut apples treated with 0.2% water chestnut extracts increased by 15% and 60.5%, respectively, compared with those in apples treated with the control. The activity of polyphenol oxidase decreased by 50%. Total colony count and weight loss significantly decreased. The results of this work indicated that treatment with water chestnut peel extracts may be a promising method for the preservation of fresh-cut apples.

Keywords: Water chestnut, peel extracts, preservation

Introduction
China has abundant water chestnut resources, and water chestnut skins account for 20% to 25% of fresh water chestnuts. Studies have found that water chestnut skins contain high amounts of bioactive substances, such as pigments, polysaccharides, polyphenols and flavonoids [1-4]. However, in water chestnut processing, most of water chestnut skins are discarded as waste, except for small amounts that are used as feed. Fully utilising these wastes can be will be of importance for increasing the added value of water chestnuts. The extracts of water chestnut skins has antibacterial activity [5-7] and antioxidant activity [8] and effectively extended the shelf life of numerous foods, such as dough [9] pork [10] and banana [11]. The use of low-cost water chestnut skins as raw materials for natural plant food preservatives reduces environmental pollution, provides a new way for waste treatment and broadens the types of natural preservatives and antioxidants available.
Apple is known as the king of fruit in China. It is used as a weight loss food by many people, who eat one apple every day. Fresh-cut apples are favoured by consumers because of their freshness and convenience. However, the mechanical damage caused by the peeling and cutting of fresh-cut apples leads to the destruction of cell structure and then causes problems, such as browning, tissue ageing, water loss and softening, which reduce nutrition and flavour and severely shorten the shelf life of fresh-cut apples.

Materials and Methods
Materials
Apples and water chestnuts were purchased from a farmers' market in Lianyungang, China.

Preparation of Water Chestnut Skin Extracts
A total of 40 g of water chestnut peels was weighed, added with a small amount of 95% ethanol, mashed into a homogenate by a juicer, mixed fully with 500 mL of 95% ethanol, extracted at 40 ℃ for 16 h and concentrated (SHZ-D III circulating water vacuum pump, Shanghai Hujia Instrument Equipment Co., LTD.) into 1 g/mL water chestnut peel extract through vacuum distillation. The resulting extract was then soaked and filtered. The residue was discarded, and the filtrate was collected and stored in a refrigerator (SC-360Y, Qingdao Aucma Co., LTD., China) at 4 ℃ for later use [12].

Fresh-cut Apple Treatment
Virus-free apples were selected; cleaned; peeled and cored; and cut into equal-sized chunks, which were randomly divided into four groups in equal quantities. Of the groups, three were soaked in 0.1%, 0.2% and 0.3% water chestnut peel extract solutions for 15 min. The control group was soaked in distilled water. The soaked apple chunks were drained and placed in fresh-keeping trays, which were sealed with polyethylene plastic wrap and refrigerated at 3 ℃. Weight loss rate, hardness, colour difference value, titratable acid content, soluble solid content and other related indices were measured every 2 days. Measurements were repeated three times.

Polyphenol Oxidase Content
A total of 2.0 g of pulp tissue sample powder was weighed, placed in a centrifuge tube, added with 10.0 mL of phosphoric acid extraction buffer and centrifuged at 4 ℃ and 12 000 × *g* for 20 min. The supernatant was the enzyme extract and determined by using a spectrophotometer (723N, Shanghai Jingke Instrument Co., LTD., China) in reference to a previously reported method. The change in absorbance at 420 nm was measured and considered as the polyphenol oxidase (PPO) activity expressed in U/g.

Total Number of Colonies
Fresh-cut apple slices were sliced, and 25 g of apples was randomly collected and placed in 225 mL of 0.85% sterile physiological saline. The sample was shaken well before gradient dilution. Next, 1 mL of the dilution solution was collected, added to agar plate counting medium and spread evenly. The plate was flipped over and incubated in a constant-temperature incubator at 37 ℃ for 48 h, and bacterial colonies were counted. Each gradient had three plates, and the experiment was repeated two times [13].

Respiration Intensity Measurement
Quantitative lye was used to absorb the carbon dioxide released by fruit respiration within a certain period of time, and the remaining alkali was titrated with acid to determine the carbon dioxide released by respiration. Respiratory intensity was obtained in mg/kg·h.

Colour Measurement
A WSC-Y automatic colour difference meter was used. Three fresh-cut apples were selected from each sample bag. The L* (whiteness), b* (yellowing) and a* (redness) values on both sides of fresh-cut apples were measured by using a colourimeter. The colour difference was calculated. ΔE was used to represent the colour change of fresh-cut apples.

Weight Loss Assay: The formula for calculating the weight loss rate using weight is as follows:

$$W(\%) = 100 \times (m_1 - m_{2)}/m_1 \quad (1)$$

In the above formula, W represents the weight loss rate (%), m_1 represents the initial fruit mass (g) and m_2 represents the fruit mass measured during storage (g).

Hardness Measurement
Three sets of samples were used to measure the surface hardness of fresh-cut apple slices before and after treatment at room temperature. Three points from each slice were measured by using a texture analyser, and the average of the results was taken. Hardness was recorded by using a texture meter (FHM-1 Takemura Electric Factory, Japan)

Titratable Acid Content Determination
The method of Xu *et al*. was used as a reference.[14] A total of 25 g of processed apples was weighed, ground and filtered. The resulting filtrate was added with deionised water until it reached a volume of 100 mL. A total of 25 mL of the solution was centrifuged, added with 3-4 drops of phenolphthalein indicator and titrated with 0.1 mol/L sodium hydroxide until it turned pale red in colour and did not fade within 1 min. The amount of sodium hydroxide solution was recorded, and the measurement was conducted twice.

$$X(\%) = 100 \times C \times (V_1 - V_2) \times F \times K/m \quad (2)$$

In the above formula, X is the titratable acid content (%), C is the calibrated concentration of sodium hydroxide (mol/L), V_1 is the volume of sodium hydroxide consumed by the titration of the test solution (mL), V_2 is the volume of sodium hydroxide consumed by blank titration (mL), F is the dilution multiple of the test solution, m is the mass of the titrated sample (g) and K is the conversion coefficient of acid (g/mmol, malic acid K = 0.067 g/mmol).

Soluble Solid Content Determination
A total of 10 g of sliced apples was weighed and ground in a mortar. The sample was filtered with gauze, and the filtrate was collected for later use. Soluble solid content was determined by using a handheld refractometer (PAL-1, Guangzhou Atago Scientific Instrument Co., LTD, China). The sample was ground and filtered. The filtrate was used for determination. Measurement was repeated three times, and the average value was taken.

Statistical Analysis
All data were collected by using Excel 2016 and plotted by using Origin 2021. The average values of the three tests were taken as the experimental results, and the significance of the differences was analysed by Duncan's multiple comparison method in IBM SPSS ($p < 0.05$).

Results and Discussion

PPO Activity
Given that PPO is the most important enzyme in the enzymatic browning of fresh-cut apples, changes in PPO indirectly reflect the browning of apple tissues. The PPO activity in fresh-cut apples firstly increased and then decreased during storage. With the increase in storage time, PPO activity in the control group gradually increased and reached the maximum value on day 6 and then slightly decreased (Fig. 1).
Throughout the whole storage process, PPO activity in the three groups was lower than that in the control group because the complexes of citric acid and metal ions. The activity of PPO was inhibited, and the enzymatic browning of fresh-cut fruits and vegetables decelerated.

Total Colony Number
The total number of colonies is an important factor affecting the quality of fresh-cut apples. The number of bacteria produced by apple samples increased with the increase in storage days.

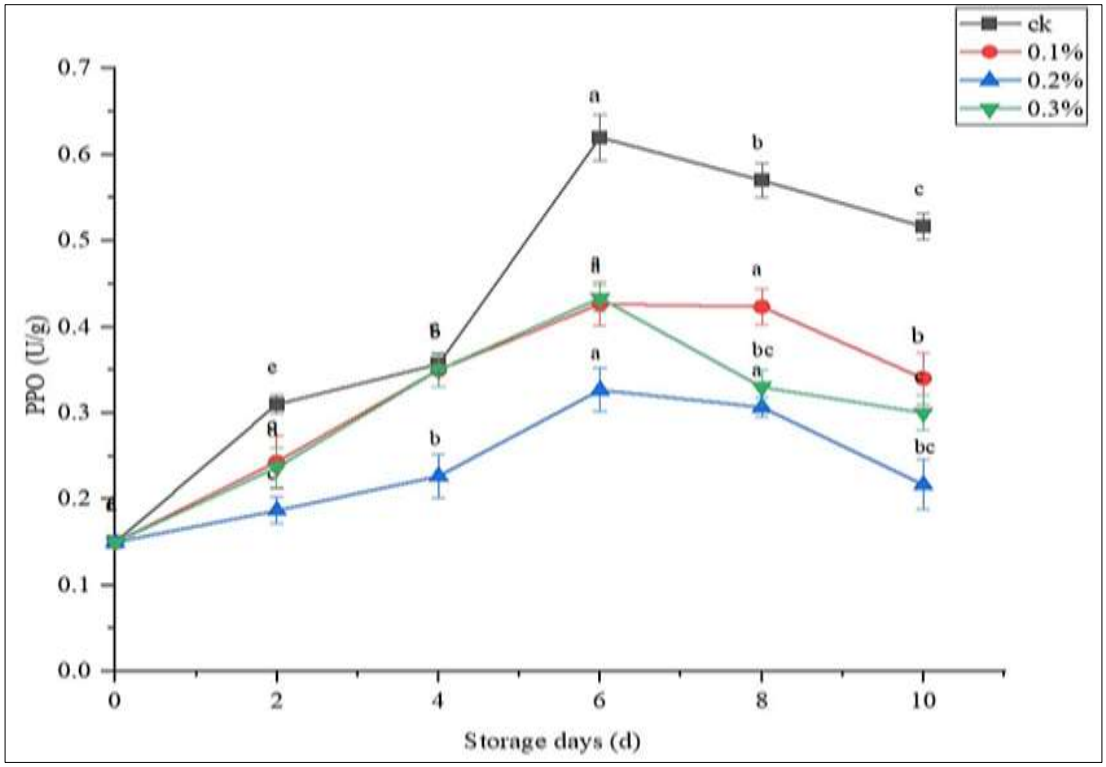

Fig 1: Effects of different concentrations of water chestnut extract on PPO of fresh-cut apples. ck: distilled water; 0.1: 0.1% Water chestnut skin extract; 0.2: 0.2% Water chestnut skin extract; 0.3: 0.3% Water chestnut skin extract; Values are the mean ± SD (n = 3). Vertical bars represent standard error. The difference between the values labelled with different alphabets at the same storage duration was statistically significant (p< 0.05, Tukey-Kramer's multiple range test).

The samples in the control group reached the critical value of edible standards on day 6 of storage, whereas those in the treatment group remained edible after day 10 (Fig. 2). The total number of colonies in the 0.2% extract treatment group was significantly lower than that in other groups, indicating that treatment with 0.2% water chestnut extract had a remarkable effect on inhibiting the growth of bacteria (Fig. 2); this could be because of the antibacterial activity of water chestnut peel extracts [7].

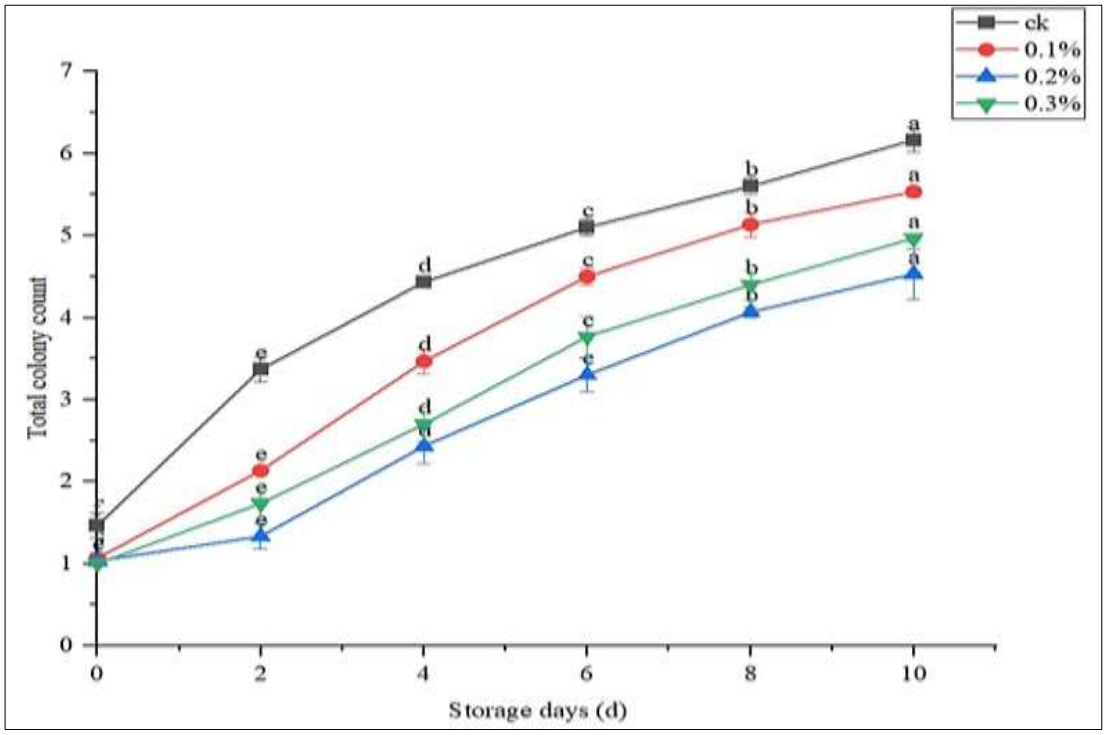

Fig 2: Effects of different concentrations of water chestnut extract on the total number of colonies of fresh-cut apples. ck: distilled water; 0.1: 0.1% Water chestnut skin extract; 0.2: 0.2% Water chestnut skin extract; 0.3: 0.3% Water chestnut skin extract; Values are the mean ± SD (n = 3). Vertical bars represent standard error. The difference between the values labelled with different alphabets at the same storage duration was statistically significant (p< 0.05, Tukey-Kramer's multiple range test).

Respiration Rate: The orderly life metabolism of fruits after harvest requires tissues to provide energy through respiration. The intensity of respiration during storage is an important index for evaluating the consumption of nutrients and senescence of fruits after harvest. High respiration intensity is indicative of strong respiration. Treatment with 0.2% water chestnut extract effectively inhibited respiratory action, reduced respiratory consumption and maintained apple quality by not interfering with normal tissue respiratory metabolism (Fig. 3).

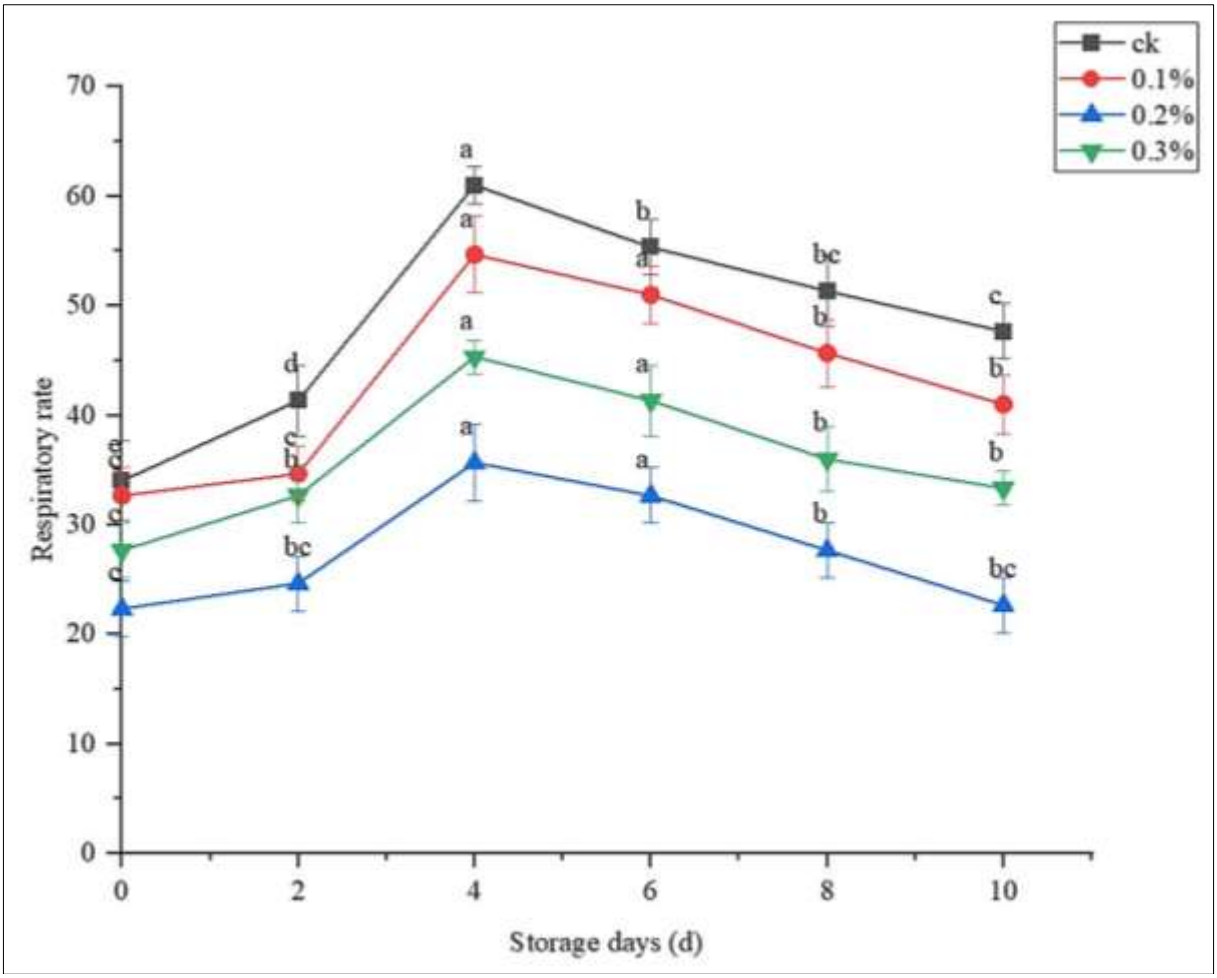

Fig 3: Effects of different concentrations of water chestnut extract on respiration rate of fresh-cut apples. ck: distilled water; 0.1: 0.1% Water chestnut skin extract; 0.2: 0.2% Water chestnut skin extract; 0.3: 0.3% Water chestnut skin extract; Values are the mean ± SD (n = 3). Vertical bars represent standard error. The difference between the values labelled with different alphabets at the same storage duration was statistically significant ($p< 0.05$, Tukey-Kramer's multiple range test).

Colour Difference

The ΔE value of apple samples decreased gradually with the increase in storage days. The decrease in the control group was the largest, whereas that in the 0.2% treatment group was gradual. The ΔE value after day 10 in the 0.2% treatment group was significantly higher than that in other treatment groups (Fig. 4), indicating that 0.2% extract can effectively inhibit the browning of fresh-cut apples.

Weight Loss

With the prolongation of time, the weight loss of fresh-cut apples in the control group increased linearly, whereas that in in the treatment group was low during 0-6 days of storage and slightly increased after 6 days of storage (Fig. 5). This finding showed that water chestnut peel extract could decelerate weight loss. Overall, 0.2% extract could reduce the weight loss of fresh-cut apples.

Hardness: Hardness reflects the degree to which apple tissues have softened. Apple fruits soften and their hardness decreases mainly due to their respiration; pectin degradation in their tissue; and water soaking before storage, which causes their dehydration and wilting. Figure 6 shows that with the extension of storage time, the pulp hardness of all experimental groups gradually decreased. The pulp hardness of the treated group was significantly higher than that of the control group. The pulp hardness of the 0.2% group was always higher than that of other treatment groups during storage. The slow decline in pulp hardness during days 0-6 of storage maintained the tissue structure of fresh-cut apples. These results showed that 0.2% citric acid treatment can better maintain the hardness of fresh-cut apples and had a better preservation effect than other treatments. As the preservative concentration increased, apple softening was delayed.

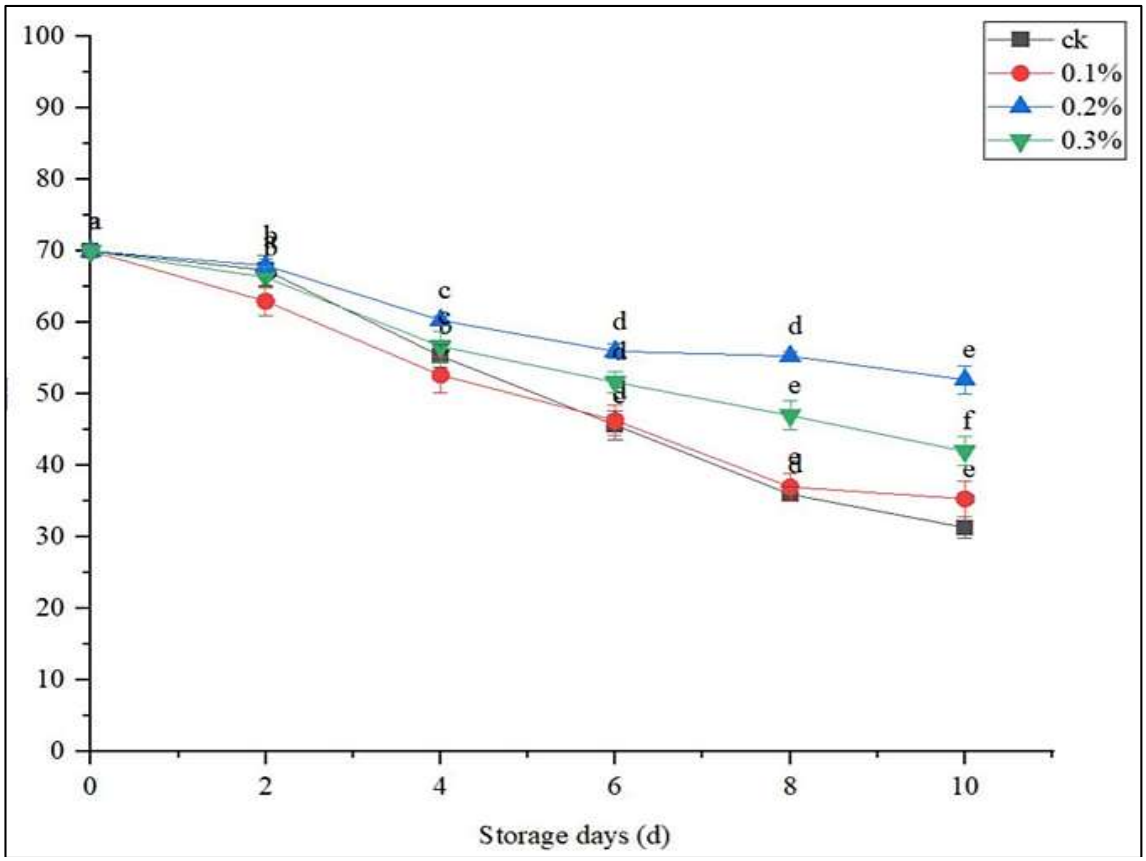

Fig 4: Effects of different concentrations of water chestnut extract on color difference of fresh-cut apples. ck: distilled water; 0.1: 0.1% Water chestnut skin extract; 0.2: 0.2% Water chestnut skin extract; 0.3: 0.3% Water chestnut skin extract; Values are the mean ± SD (n = 3). Vertical bars represent standard error. The difference between the values labelled with different alphabets at the same storage duration was statistically significant ($p< 0.05$, Tukey-Kramer's multiple range test).

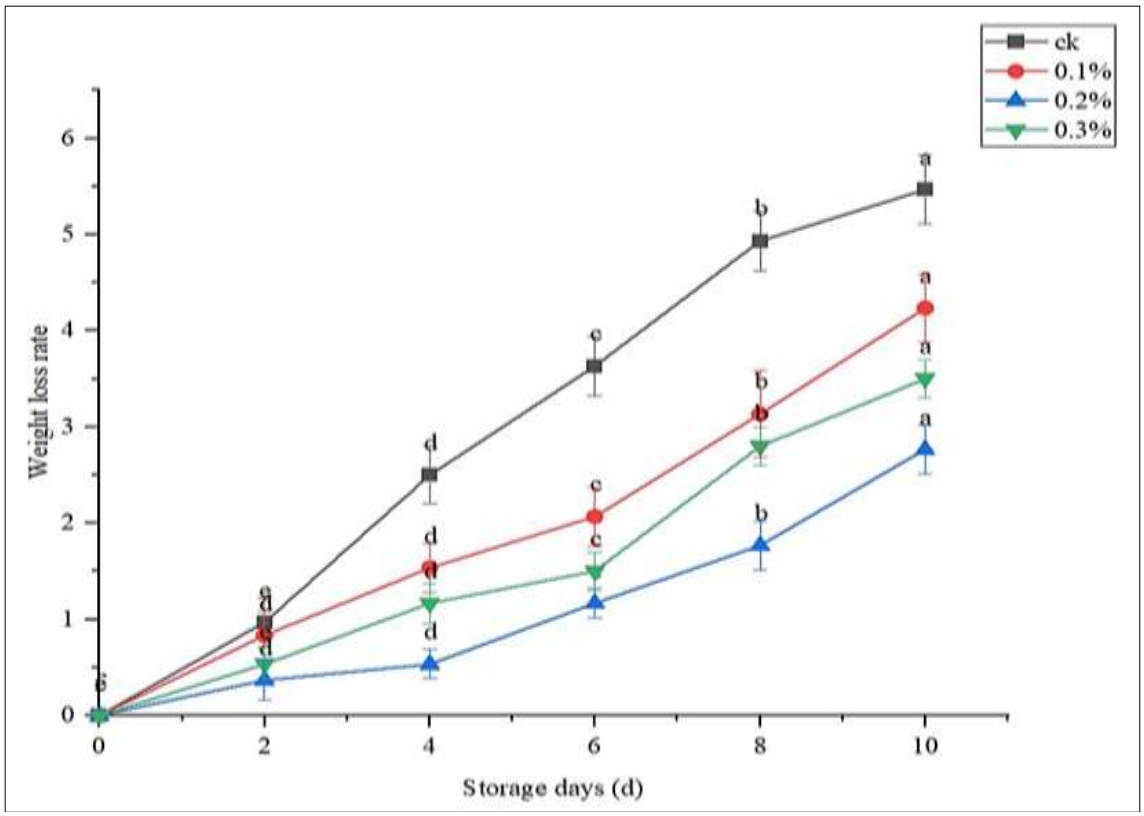

Fig. 5: Effects of different concentrations of water chestnut extract on weight loss rate of fresh-cut apples. ck: distilled water; 0.1: 0.1% Water chestnut skin extract; 0.2: 0.2% Water chestnut skin extract; 0.3: 0.3% Water chestnut skin extract; Values are the mean ± SD (n = 3). Vertical bars represent standard error. The difference between the values labelled with different alphabets at the same storage duration was statistically significant ($p< 0.05$, Tukey-Kramer's multiple range test).

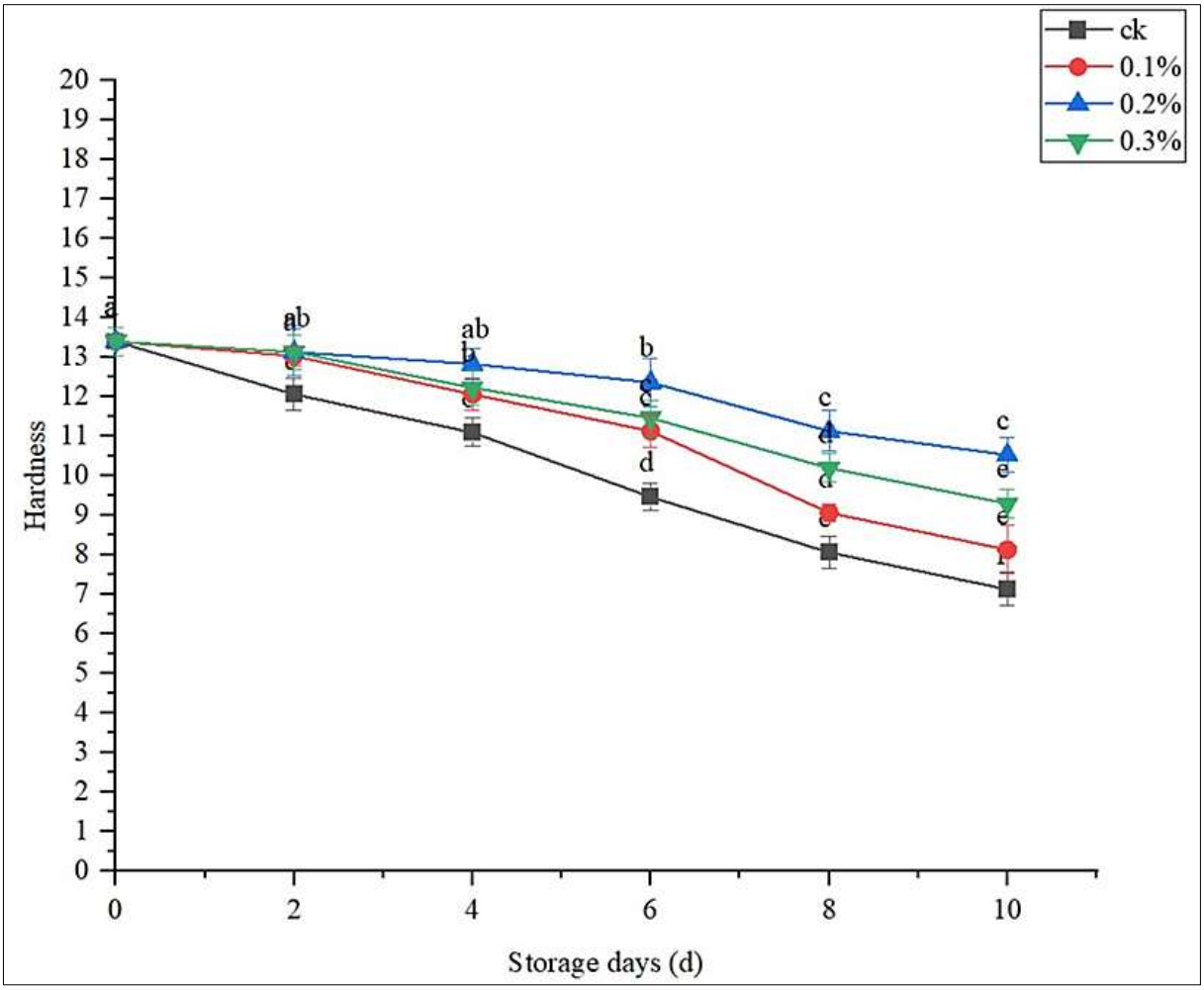

Fig 6: Effects of different concentrations of water chestnut extract on the hardness of fresh-cut apples. ck: distilled water; 0.1: 0.1% Water chestnut skin extract; 0.2: 0.2% Water chestnut skin extract; 0.3: 0.3% Water chestnut skin extract; Values are the mean ± SD (n = 3). Vertical bars represent standard error. The difference between the values labelled with different alphabets at the same storage duration was statistically significant (p< 0.05, Tukey-Kramer's multiple range test).

Titratable Acid Content: The titratable acid content of fresh-cut apples decreased, with the titratable acid reduction rate being highest in the control group and lowest in the 4 mg/mL dose group, indicating that appropriate tea polyphenol extract treatment can reduce the loss of titratable acid content during apple storage (Fig. 7). Amongst all groups, the 0.2% and 0.3% water chestnut skin treatment groups had the highest titratable acid contents. The titratable acid content in the other experimental groups increased. In general, the change in titratable acid content during storage is complicated (Fig. 7). Titratable acid content increased could be due to the increase in water loss rate. Therefore, it increased at the early stage of storage. At the end of storage, microorganisms in fresh-cut apple slices had accumulated a certain amount of acidic substances with the rapid increase in decay rate. This phenomenon also led to a remarkable change in the titratable acid content of fresh-cut apples. In addition, respiration consumed organic acids, reducing the titratable acid content of fresh-cut apples. At the end of storage, the water loss rate and organic acid consumption by respiration in each experimental group reached their maximum values. Therefore, the change in the titratable acid content of fresh-cut apples in each experimental group is complicated [15].

Soluble Solid Contents

The soluble solid content in all experimental groups decreased with the extension of storage time mainly because apples were still conducting life activities during storage. soluble solid was constantly consumed during this process, resulting in a gradual decline in its content (Fig. 8). The soluble solid content in the treatment group was higher than that in the control group starting on day 2. The soluble solid content in the 0.2% treatment group was the highest and was higher than that in other experimental groups after day 2 (Fig. 7). These results showed that water chestnut extract maintained the soluble solid content of fresh-cut apples and that amongst all treatments, treatment with 0.2% water chestnut extract had the best preservation effect, which delayed nutrient loss in fresh-cut apples.

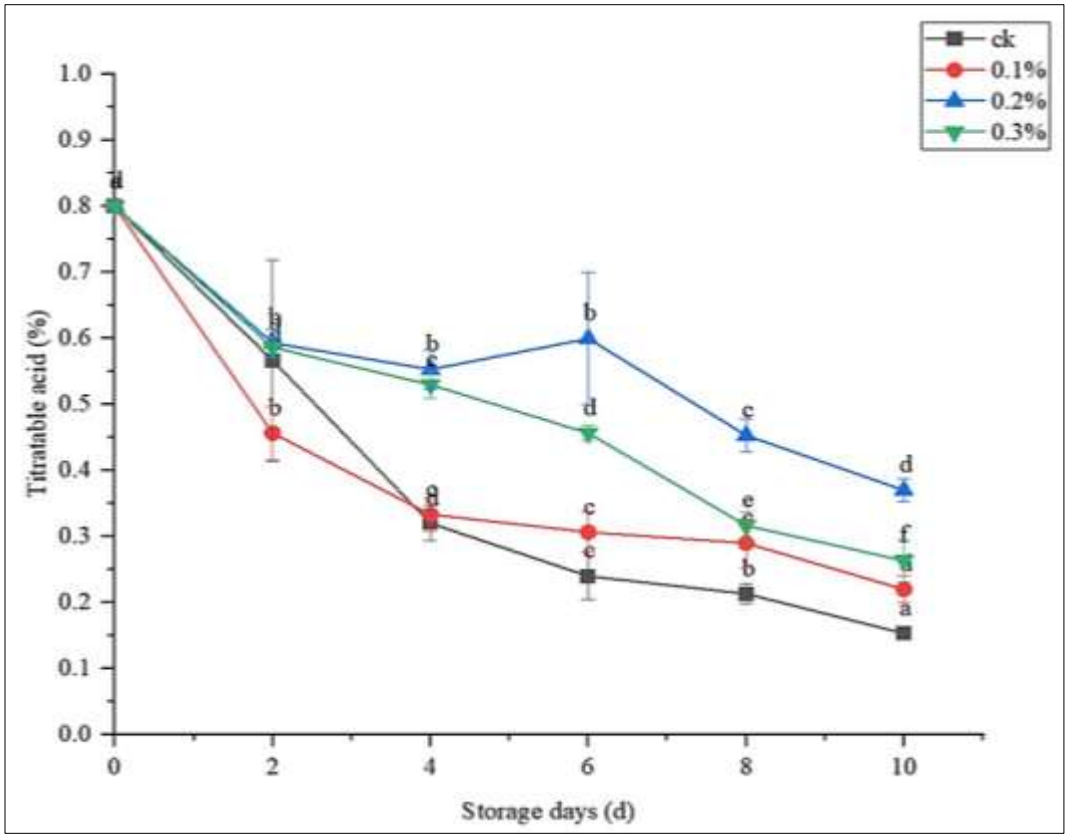

Fig 7: Effects of different concentrations of water chestnut extract on titratable acid of fresh-cut apples. ck: distilled water; 0.1: 0.1% Water chestnut skin extract; 0.2: 0.2% Water chestnut skin extract; 0.3: 0.3% Water chestnut skin extract; Values are the mean ± SD (n = 3). Vertical bars represent standard error. The difference between the values labelled with different alphabets at the same storage duration was statistically significant ($p< 0.05$, Tukey-Kramer's multiple range test).

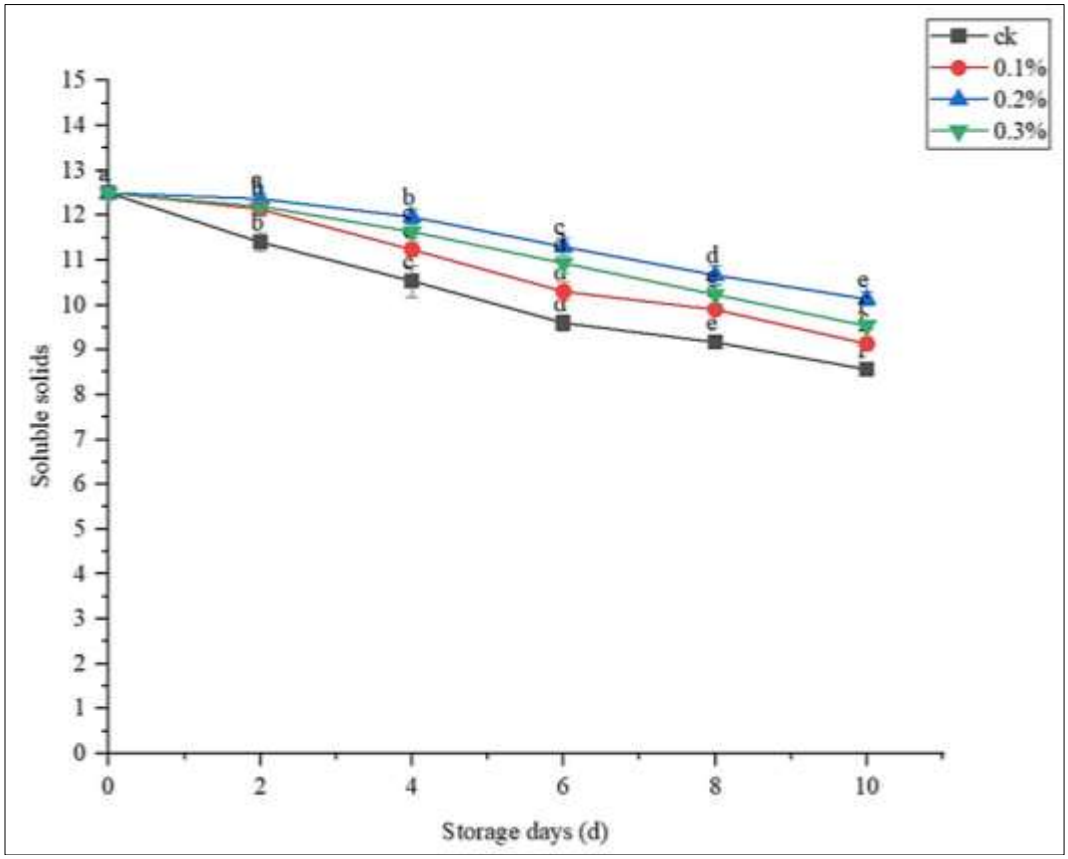

Fig 8: Effects of different concentrations of water chestnut extract on soluble solids content of fresh-cut apples. ck: distilled water; 0.1: 0.1% Water chestnut skin extract; 0.2: 0.2% Water chestnut skin extract; 0.3: 0.3% Water chestnut skin extract; Values are the mean ± SD (n = 3). Vertical bars represent standard error. The difference between the values labelled with different alphabets at the same storage duration was statistically significant ($p< 0.05$, Tukey-Kramer's multiple range test).

Conclusions

The effect of water chestnut extract on fresh-cut apples was analysed in this experiment. The results showed that 0.2% water chestnut extract could inhibit the activity of PPO and growth of total colonies in fresh-cut apple tissues. The decrease in hardness, weight loss rate, Vc and soluble solid content was delayed, thus improving the preservation of fresh-cut apples.

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