The Preservation Effect of Ascorbic Acid and Calcium Chloride Modified Chitosan Coating on Fresh-cut Apples at Room Temperature

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Abstract: The consumption of fresh-cut apples has grown rapidly due to consumers' increasing willingness to enjoy prepared and ready-to-eat fruits. But fresh-cut apples tend to experience severe enzymatic browning which discourages people to taste. Chitosan (CS) coating containing ascorbic acid (AA) or calcium chloride (CaCl₂) were applied on fresh-cut apples in this work to observe their preservation effects. Their appearance, weight change were evaluated during the storage at room temperature, besides, AA content, soluble solid content were analyzed at the end of storage. CS-CaCl₂ coating has better preservation effect than CS-AA coating when they were applied on fresh-cut apples. CS-CaCl₂ coating showed lower weight loss and higher AA content of apple slices than CS-AA coating. CS-CaCl₂ coating was also good at anti-browning in terms of the appearance of coated apple slices.

Keywords: Fresh-cut apples; Preservation; Room Temperature; CaCl₂; CS

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1 Introduction

Fruits are good sources of vitamins, minerals and fibers, moreover, they have become an important segment of our daily life. Apple helps improving people's health because of their various nutritional constituents such as vitamins and phenolic compounds [1]. Nowadays, more and more apples are cut into pieces for the convenience of consumers. In food service establishments, school lunch programs and our own house, fresh-cut apples have recently emerged as popular snacks [2]. However, fresh-cut apples are susceptible to be spoiled because of their enzymatic browning. And the most remarkable negative effect of enzymatic browning is the color deterioration which is the main reason of impairing fruit quality and discouraging consumers to purchase fresh-cut apples [3, 4, 5]. Thus, it is an urgent task to develop proper methods to reduce the decay of fresh-cut apples and prolong their shelf-life. Nowadays, fresh-cut apples are typically stored under refrigerated conditions to maintain their quality. However, after being stayed in a cold circumstance for a long time, apple slices may suffer from physiological injury. Moreover, this method is costly and energy consuming. Edible coatings have become a method that is a potential for the development of high-quality fresh-cut fruits [6][7]. Poverenov et al. reported that the LBL alginate–chitosan coating was found to possess the beneficial properties of both ingredients and antimicrobial activity [8]. The data of Xiao et al. suggested that chitosan incorporated with rosemary improved the antioxidant protection and sensory qualities of fresh-cut pears and showed the antibrowning ability [9]. Qi et al. demonstrated coatings in combination with anti-browning agents (ascorbic acid and CaCl_2) effectively retarded enzymatic browning on minimally processed apples during storage and retarded or avoided tissue softening of the apple slices [10].

CS produced by the deacetylation of chitin [11] is cationic, nontoxic, biodegradable, biocompatible and has the film-forming ability as well as antifungal effects on various plant pathogenic bacteria and fungi [12]. In previous studies, CS was used to preserve fruit quality and performed well [13, 14, 15]. Employing CaCl_2 on the preservation of apples is an important approach to avoid browning with the modified (reduced O_2) atmospheres [16]. Souza et al. (2015) [17] represented that adding CaCl_2 into CS coating showed good preservation effects on strawberries in terms of firmness, weight loss and microbial growth. It has been reported that AA, a nutrient that is needed for consumers, is effective in controlling enzymatic browning. Tortoe et al. (2007) [18] observed moderate anti-browning effects on ‘Golden Delicious’ apple slices using AA for up to 14 days when apple slices were stored at 4 °C. Son et al. (2001) [19] also reported the effect of AA on anti-browning of fresh-cut apples. Nontoxic materials such as AA and calcium source have been proposed as excellent treatments for fresh-cut apples to inhibit enzymatic browning [19, 20, 21]. However, these methods have limited success in extending the shelf-life of fresh-cut apples because of low efficiency and off flavors [3, 4]. Although CS has great film-forming ability, CS film’s fruit preservation effect is relatively low. Thus, this work was undertaken to research the effect of CS coating modified with CaCl_2 or AA on prolonging the shelf-life of fresh-cut apples at room temperature.

2 Materials and methods

2.1 Materials

Ascorbic acid was obtained from Sinopharm Chemical Reagent Co., LTD (Shanghai, China). Calcium chloride (food grade) was purchased from Zhejiang Dacheng Calcium Industry Co., LTD (Zhejiang, China). Phosphate buffer (pH=8.0)

was provided by Jiangsu KeyGen Biotechnology Co., LTD and potassium bromide (KBr) was from Shanghai ShiSiHeWei Chemical Co., LTD. Chitosan ($M_w=590000$, D.D: 80-95%) was supplied by Sinopharm Chemical Reagent Co., LTD (Shanghai, China).

2.2 Preparation of apple samples

“Fuji” apples that were uniformity of size, color and ripeness and didn’t have apparent injury or infection were selected from a local wholesale distributor (Jiangsu, China). Apple were pre-washed in 500 ppm chlorinated water for 2 min to reduce the natural flora, and then they were washed thoroughly with sterile water to remove the residual chlorine. Apples were then cut into apple slices with a sterile sharp knife on a sterile cutting board. Besides, the apple slices that were without peel and core were similar in size and shape (their weight were 8 ± 0.5 g)

2.3 Packaging and storage of fresh-cut apples

Packaging of apple slices was carried out as described in [22] with some modification. The CS- CaCl_2 solution was prepared by mixing 100 mL 4 mg/mL CS solution with 100 mL 4 mg/mL CaCl_2 solution. CS-AA solution was prepared in the same way as preparing CS- CaCl_2 solution but CaCl_2 was took place by AA. Prepared apple slices were randomly divided into two groups and they were then dipped in the 200 mL CS- CaCl_2 and CS-AA solution separately for 5 min. Afterwards, the apple slices were drain at room temperature for 2 min and then randomly placed in a $20.7 \text{ cm} \times 14.5 \text{ cm}$ melamine tray. The control apple slices were tackled with ultrapure water instead. Images were taken at 0, 2, 4, 6, 8 and 10 h (the drying time of 2 min was set as 0 h) by an optical camera after apple slices had been picked up from their

solutions. The tested apple slices were put on a piece of white paper the moment they were being taken photos to exclude the disturbance of other substance.

2.4 Measurement of weight loss

It's assumed that weight loss is entirely corresponded with water loss which is a vital factor of evaluating apple quality. Moreover, the increase of water loss has a great impact on the appearance of apples. To determine the water loss inhibition effect of all the tested coatings, the weight of all the apple slices was individually monitored during the storage time with an analytical balance (Shanghai Precision Scientific Instrument Co., LTD. Shanghai, China) at 0, 2, 4, 6, 8, 10 h.

The weight loss ratio W (%) was expressed as:

$$W(\%) = \frac{m_i - m_t}{m_i} \times 100\%$$

where m_i was the initial weight and m_t was the weight at the end of storage.

2.5 Measurement of AA content

At the end of storage time, apple slices were grinded in 40 mL 0.1 mol/L phosphate buffer with glass mortar. All the substance in the mortar was transferred into a 50 mL centrifuge tube and the volume was adjusted into 50 mL with ultrapure water. After that, the mixture was centrifuged at 12000 rpm for 30 min at 4 °C using laborzentrifugen centrifugal machine (3-30 K, Sigma, Osterode am Harz, Germany). The supernatant was filtered using Whatman No. 1 filter paper. We set apart 20 mL supernatant to measure the soluble solid content. AA content was obtained by the absorbance of the supernatant at 243 nm (A_{243}) according to [23, 24]. The equipment used was a dual beam UV and visible spectrophotometer (a-1900 PC, Shanghai Aoxi

Scientific Instrument Co., LTD. Shanghai, China). The result was expressed as the mean of the three replication of the absorbance value.

2.6 Soluble solid content

SSC was conducted at these supernatant samples according to [25] with some modification using a Abbe refractometer (2WAJ, Shanghai YuGuang Instrument Co., LTD, Shanghai, China). This measurement was processed at room temperature (25 ± 2 °C).

SSC was represented as reflective index, meanwhile, ultrapure water was set as the base line of those tested samples' reflective index.

2.7 Fourier transform infrared spectroscopy (FT-IR) analysis

After the experiments stated above, we found that CS-CaCl₂ coating showed better anti-browning and quality maintaining ability. Thus, FT-IR analysis of CS-CaCl₂ coating was conducted to analysis the possible reasons of its preservation of fresh-cut apples. CS-CaCl₂ mixture solution was prepared as stated in 2.3 and three drops of the solution were dipped on a glass. After that, the glass sheet was dried at 60 °C for 24 h to prepare the dried CS-CaCl₂ coating. Dried CS-CaCl₂ coating and pure CS powder were used for FT-IR spectra analysis. 2 mg CS-CaCl₂ coating sample was scraped from the glass. The tested samples (CS-CaCl₂ coating and pure CS powder) were pressed into a disc with 200 mg of KBr to gain the FT-IR spectra. FT-IR was recorded to analysis their chemical structure. The measurement of FT-IR spectra was carried out with a Nicolet Nexus-470 Fourier transform infrared spectrometer (Thermo Nicolet, USA) at the wavenumber region of 4000-400 cm⁻¹ using 32 scans.

3 Results and discussions

3.1 Appearance of apple slices

Browning of fruits was the result of oxidation processes that occurred at advanced maturation stages [26]. As shown in Fig. 1, great changes have taken place on coated apple slices after a period of time, and all of the slices became brown more or less. All the coatings were found adhering tightly to the surface of the apple slices during the entire storage time. The anti-browning effect of CS-AA coating on fresh-cut apples was not very apparent and the browning degree of slices coated with CS-AA coating was similar to the degree of the control apple slices. CS-CaCl₂ coating helped maintaining the whiteness of apple slices as time went on and thus could be acted as a good anti-browning agent. Meanwhile, the color of apple slices coated with CS-CaCl₂ coating was greatly lighter than the others at the end of storage time. This phenomenon indicated that the anti-browning ability of CS-AA coating was weaker than CS-CaCl₂ coating.

Fig. 1. Appearance images of fresh-cut apples after coated with (a) ultrapure water (control group), (b) CS coating, (c) CS-AA coating and (d) CS-CaCl₂ coating at 0 , 2, 4, 6, 8 and 10 h.

3.2 Weight loss of apple slices

Weight loss occurred mainly due to water loss, as Olivas and Barbosa-Cánovas [27] stated, because the other components that would be lost (e.g. gaseous products of respiration) are practically undetectable in terms of weight loss. The weight of coated fresh-cut apples was detected to analyze the efficiency of these coatings acting as water loss inhibitor over the experimental time. As time went on, weight of apple slices decreased gradually (Fig. 2). Over the experimental storage period, the variation trend of all the apple slices was similar. All the coated apples demonstrated their good ability of water loss inhibition when compared with the control apple slices. The

weight loss ratio of apple slices coated with nothing was more than 15% while the others were less than 14%. Moreover, CS-CaCl₂ coating showed the best performance of water loss inhibition because apple slices coated with CS-CaCl₂ coating had the lowest weight loss ratio of about 11%. This finding was in agreement with a report of Souza et al. (2015) [17] who represented that the addition of CaCl₂ into CS coating had the least loss of coated strawberries' weight.

Fig. 2. Weight change of fresh-cut apples during the storage time and the fresh-cut apples are coated with CS, CS-AA and CS-CaCl₂ coating respectively. Weight loss ratio of 10 h is shown on the top right corner. Vertical bars represent standard deviation of the means (n = 3).

3.2 AA content of apple slices

AA is one of the most important nutrient in fruits and it has many biological activities in human body [28]. Changes in AA content of sliced apples are shown in

Fig. 3. All the tested apple slices' AA content was higher than that of the control apple slices and this finding indicated that all the coatings possessed the ability to maintain AA content of apple slices. Both CS-AA coating and CS-CaCl₂ coating enhanced the inhibition of AA loss when compared with CS coating. Obviously, CS-CaCl₂ coating performed best and the A₂₄₃ reached the value of 1.25. All the findings in terms of AA content showed that both CS-AA coating and CS-CaCl₂ coating had the ability to maintain the nutritional ingredient of apple slices.

Fig. 3. Absorbance of apple slices' supernatant at 243 nm which represents AA content (mean $\pm$ SD, $n=3$).

3.4 Soluble solid content of apple slices

Coating imposes a reduction in the metabolic activity of fruits and leads to a slowdown of polysaccharide degradation reactions and, therefore, to a lower SSC value of the coated fruits [24]. That is to say, the lower SSC, the stronger inhibition of

fruit decay. The SSC of apple slices coated with CS-AA coating was lower than those of other coated apples. However, as shown in Fig. 4, all the tested apple slices' SSC were higher than the control apples' SSC and this finding indicated that CS-AA coating and CS-CaCl₂ coating might lack the ability to restrain metabolic activity of fresh-cut apples.

Fig. 4. SSC of coated fresh-cut apples after they have been stored at room temperature (25 ± 2 °C) for 10 h. The reflective index of ultrapure water is set as the baseline and vertical bars represent the standard deviation of the means ($n = 3$).

3.5 FT-IR analysis and molecular structure of CS-CaCl₂ coating

FT-IR analysis was described in many references as the way to investigate the interaction between different substance [29, 30, 31]. In this study, dried CS-CaCl₂ coating and pure CS powder were analyzed by FT-IR to observe the possible change of CS-CaCl₂ coating when it was compared with CS. A broad band of CS could be

observed at 3700-3100 cm^{-1} because of the stretching vibration of $-\text{NH}_2$ and $-\text{OH}$ groups [32, 33] and the strong intermolecular hydrogen bond broaden the peak width. There still existed $-\text{C}=\text{O}-\text{NHR}$ in CS because of the surplus of acetyl. CS exhibited the main characteristic bands of $-\text{C}=\text{O}-\text{NHR}$ I and II at 1649 cm^{-1} and 1595 cm^{-1} respectively (Fig. 5a). The peak of $-\text{C}=\text{O}-\text{NHR}$ I shifted to 1641 cm^{-1} and $-\text{C}=\text{O}-\text{NHR}$ II shifted to 1533 cm^{-1} when CS was mixed with CaCl_2 . Moreover, the band range of CS- CaCl_2 coating near 3500 cm^{-1} was narrower than CS which probably because CaCl_2 influenced the hydrogen bond of CS (Fig. 5b). Once added to the CS solution, CaCl_2 might form a pair of solvent separation electronic in the mixed solution which formed coordination complex with $-\text{OH}$ [34]. This observation showed the possibility of a weak interaction between CaCl_2 and CS which might be the reason of the anti-browning and nutritional ingredient maintaining ability of CS- CaCl_2 coating. The preservation of apple slices might because of the contribution of Ca^{2+} to the linkages between pectic substances within the cell-wall [35]. This reaction was enhanced by CS in apple slices coated with CS- CaCl_2 coating because CS showed great film-forming ability.

Fig. 5. (a) FT-IR spectra of CS powder and CS-CaCl₂ coating at the wavenumber ranging from 4000 cm^{-1} to 400 cm^{-1} ; (b) The molecular structure of CS-CaCl₂ coating and this analyses is performed using ChemBioDraw Ultra 12.0.

4 Conclusions

The purpose of this work was to investigate the effect of CS-AA coating and CS-CaCl₂ coating on the anti-browning and quality maintaining of fresh-cut apples. All the coatings were found adhering tightly to the surface of the apple slices during the entire storage time. All the coatings possessed the ability to maintain AA content of apple slices. CS was not only a good anti-browning agent but also a good nutrient

holder when it was mixed with CaCl_2 . Concluding from the lower weight loss and higher AA content of apple slices, CS- CaCl_2 coating showed a great quality maintaining ability when compared with CS-AA coating. But CS- CaCl_2 coating seemed to lack the ability of inhibiting the metabolic activity of fresh-cut apples as the SSC of apple slices coated with CS- CaCl_2 coating was not so reasonable. In this work, we have presented an effective and low-cost method to enhance the preservation of fresh-cut apples. This method could largely prolong the shelf-life of fresh-cut apples at room temperature and don't produce any negative changes in sensory attributes. We hope that this method will be a new system for food preservation and will have lots of possible industrial applications.

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References

- [1] E. Biedrzycka., R. Amarowicz, Diet and health: apple polyphenols as antioxidants. Food Rev. Int. 24 (2008) 235-251.
- [2] J.R. Gorny, New opportunities for fresh-cut apples. Fresh Cut 11 (2003) 14-15.

- [3] H.P.V. Rupasinghe, D.P. Murr, J.R. DeEll, J. Odumeru, Influence of 1-methylcyclopropene and nature seal on the quality of fresh-cut “empire” and “crispin” apples. *J. Food Quality* 28 (2005) 289-307.
- [4] S. Lu, Y. Luo, E. Turner, H. Feng, Efficacy of sodium chlorite as an inhibitor of enzymatic browning in apple slices. *Food Chem.* 104 (2007) 824-829.
- [5] C. Severini , A. Baiano , T.D. Pilli, R. Romaniello, A. Derossi, Prevention of enzymatic browning in sliced potatoes by blanching in boiling saline solutions. *Lebensmittel-Wissenschaft und-Technol.* 36 (2003) 657-665.
- [6] M.R. Moreira, L. Cassani, O. Martín-Belloso, R. Soliva-Fortuny, Effects of polysaccharide-based edible coatings enriched with dietary fiber on quality attributes of fresh-cut apples. *J. Food Sci. Technol.* 52 (2015) 7795-7805.
- [7] Y. Ruan, M. Hu, J. Fan, L. Zhang, Effect of the cantaloupe-cutting soaked with calcium chlorine on its fresh-keeping. *J. Aihui Agri. Sci.* 34 (2006) 6434-6435.
- [8] E. Poverenov, S. Danino, B. Horev, R. Granit, Y. Vinokur, V. Rodov, Layer-by-layer electrostatic deposition of edible coating on fresh cut melon model: anticipated and unexpected effects of alginate–chitosan combination. *Food Bioprocess Tech.* 7 (2014) 1424-1432.
- [9] C. Xiao, W. Luo, M. Liu, L. Zhu, M. Li, H. Yang, Y. Deng, Quality of fresh-cut pears (*Pyrus bretschneideri* Rehd cv. Huangguan) coated with chitosan combined with ascorbic acid and rosemary extracts. *Philipp. Agri. Sci.* 93 (2010) 66-75.
- [10] H. Qi, W. Hu, A. Jiang, M. Tian, Y. Li, Extending shelf-life of Fresh-cut ‘Fuji’ apples with chitosan-coatings. *Innov. Food Sci. Emerg.* 12 (2011) 62-66.

- [11] M. Rinaudo, Chitin and chitosan: properties and applications. *Prog. Polym. Sci.* 31 (2006) 603-632.
- [12] H. Zhang, R. Li, W. Liu, Effects of chitin and its derivative chitosan on postharvest decay of fruits: a review. *Int. J. Mol. Sci.* 12 (2011) 917-934.
- [13] H. Arnon, Y. Zaitsev, R. Porat, E. Poverenov, Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. *Postharvest Biol. Tec.* 87 (2014) 21-26.
- [14] C. Han, J. Zuo, Q. Wang, L. Xu, B. Zhai, Z. Wang, H. Dong, L. Gao, Effects of chitosan coating on postharvest quality and shelf life of sponge gourd (*Luffa cylindrica*) during storage. *Sci. Hortic-Amsterdam* 166 (2014) 1-8.
- [15] K. Liu, C. Yuan, Y. Chen, H. Li, J. Liu, Combined effects of ascorbic acid and chitosan on the quality maintenance and shelf life of plums. *Sci. Hortic-Amsterdam* 176 (2014) 45-53.
- [16] E. Aguayo, C. Requejo-Jackman, R. Stanley, A. Woolf, Effects of calcium ascorbate treatments and storage atmosphere on antioxidant activity and quality of fresh-cut apple slices. *Postharvest Biol. and Tec.* 57 (2010) 52-60.
- [17] M.P. Souza, A.F.M. Vaz, H.D. Silva, M.A. Cerqueira, A.A. Vicente, M.G. Carneiro-da-Cunha, Development and Characterization of an Active Chitosan-Based Film Containing Quercetin. *Food Bioprocess Tech.* 8 (2015) 2183-2191.
- [18] C. Tortoe, J. Orchard, A. Beezer, Prevention of enzymatic browning of apple cylinders using different solutions. *Int. J. Food Sci. Technol.* 42 (2007) 1475-1481.

- [19] S.M. Son, K.D. Moon, C.Y. Lee, Inhibitory effects of various antibrowning agents on apple slices. *Food Chem.* 73 (2001) 23-30.
- [20] M.A. Rojas-Grau, A. Sobrino López, M.S. Tapia, O. Martin Belloso, Browning inhibition in fresh-cut “Fuji” apple slices by natural antibrowning agents. *J. Food Sci.* 71 (2006) 559-565.
- [21] X. Fan, B.A. Niemera, J.P. Mattheis, H. Zhuang, D.W. Olson, Quality of fresh-cut apple slices as affected by low-dose ionizing radiation and calcium ascorbate treatment. *J. Food Sci.* 70 (2006) 143-148.
- [22] F. Tardelli, L. Guidi, R. Massai and P.M. Toivonen, Effects of 1-methylcyclopropene and post-controlled atmosphere air storage treatments on fresh-cut Ambrosia apple slices. *J. Sci. Food Agr.* 93 (2013) 262-270.
- [23] Y. Liu, X. Sun, Q. Yang. Improvement of ultraviolet spectrophotometry to measure the content of Vc. *J. Aihui Agri. Sci.* 39 (2011) 13270-13272.
- [24] S. Supapvanich, J. Pimsaga, P. Srisujan, Physicochemical changes in fresh-cut wax apple (*Syzygium samarangense* [Blume] Merrill & L.M. Perry) during storage. *Food Chem.* 127 (2011) 912-917.
- [25] L. Abugoch, C. Tapia, D. Plasencia, A. Pastor, O. Castro-Mandujano, L. López and V.H Escalona, Shelf-life of fresh blueberries coated with quinoa protein/chitosan/sunflower oil edible film. *J. Sci. Food Agr.* 96 (2016) 619-626.
- [26] B.G.S. Medeiros, A.C. Pinheiro, J.A. Teixeira, A.A. Vicente, M.G. Carneiro-da-Cunha, Polysaccharide/protein nanomultilayer coatings: construction, characterization and evaluation of their effect on ‘Rocha’ pear (*Pyrus communis* L.) shelf-life. *Food Bioprocess Tech.* 5 (2012) 2435-2445.

- [27] G.I. Olivas, G.V. Barbosa-Cánovas, Edible coatings for fresh-cut fruits. *Crit. Rev. Food Sci.* 45 (2005) 657-670.
- [28] S.K. Lee, A.A. Kader, Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Tec.* 20 (2000) 207-220.
- [29] T. Imai, S. Shiraishi, H. Saito, M. Otagiri, Interaction of indomethacin with low molecular weight chitosan, and improvements of some pharmaceutical properties of indomethacin by molecular weight chitosans. *Int. J. Pharm.* 67 (1991) 11-20.
- [30] L. Vachoud, N. Zydowicz, A. Domard, Formation and characterization of a physical chitin gel. *Carbohydr. Res.* 302 (1997) 169-177.
- [31] W. Liu, S. Sun, Z. Cao, X. Zhang, K. Yao, W.W. Lu, K.D.K. Luk, An investigation on the physicochemical properties of chitosan/DNA polyelectrolyte complexes. *Biomaterials* 26 (2005) 2705-2711.
- [32] X. Wang, Y. Du, H. Liu, Preparation, characterization and antibacterial activity of chitosan-Zn complex. *Carbohydr. Polym.* 56 (2004) 21-26.
- [33] Y. Xu, Y. Du, Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles. *Int. J. Pharm.* 250 (2003) 215-226.
- [34] Y. Chen, X. Wu, F. Li, Infrared spectrometric study on solvation property of methanolic solution of calcium chloride. *Phys. Test. Chem. Anal.* 44 (2008) 310-313.
- [35] A. Akhtar, N.A. Abbasi and A. Hussain, Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. *Pak. J. Bot.* 42 (2010) 181-188.

The molecular structure of CS-CaCl₂ coating