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BRIEF COMMUNICATION

Layer-by-Layer Electrostatic Deposition of Edible Coatings for Enhancing the Storage Stability of Fresh-Cut Lotus Root (*Nelumbo nucifera*)

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Abstract
Various strategies have been employed to improve the storage stability of fresh-cut lotus root, which is prone to undesirable changes such as enzymatic oxidation. In our research, we targeted the formulation of polysaccharide-based edible coatings with different ionic charges from natural gum sources, such as guar gum, xanthan gum, and chitosan, due to the limited information regarding its application as coating material for fresh-cut lotus root. We have also compared the effect of single layer coatings versus layer-by-layer deposition as edible coating techniques for fresh-cut lotus root. Our results have shown that layer-by-layer coatings consisting of xanthan gum and chitosan were the most effective among all treatments, thereby reducing whiteness color (L^*) changes and weight loss up to 60% and 86%, respectively. Overall, the layer-by-layer coating technique was more effective than single layer coating, which shows a promising strategy to increase the storage stability of fresh-cut lotus root especially during transport and distribution.

Keywords Edible coating · Layer-by-layer deposition · Stability · Fresh-cut · Enzymatic browning

Introduction

The lotus root (*Nelumbo nucifera*), a vegetable grown in various countries such as Japan, Korea, China, and Vietnam, is one of the examples of the fresh-cut products that is gaining interest from researchers and industries as a novel minimally processed vegetable (Tu et al. 2015). This rhizome has a white color, crisp texture, pleasant aroma and is an excellent source of important minerals and nutrients (Gao et al. 2017). However, fresh-cut lotus root is prone to enzymatic browning, which deteriorates its quality and shortens its shelf life. Few attempts by researchers to improve the shelf life of the fresh cut lotus root have been reported. Zhou et al. (2018) investigated the effects of konjac glucomannan coating on improving the shelf-life of fresh-cut lotus root and the coating application resulted to inhibited respiration rates and tissue browning and delayed decrease of total soluble contents. Jianglian and Shaoying (2013) reported the positive effect of chitosan coating alone and chitosan coating with phytic acids in decreasing weight loss, delaying browning, and maintaining vitamin C contents in fresh-cut lotus root during storage. Therefore, the authors recognized the limited information on the application of gum sources (xanthan and guar gum) and chitosan on fresh-cut lotus root as edible coatings without the combination of any other preservation methods such as antibrowning agents and vacuum packaging. Moreover, there were no reported studies yet on the comparison of the efficacy of these edible coatings when applied as single and bilayers.

Edible coatings are usually applied to food products through single layer applications. For instance, the use of single layers of chitosan, sodium chloride, and pullulan was found to inhibit peroxidase (POD) enzyme activity and slowed the decrease of total phenolic and flavonoid contents in pears (Kou et al. 2014). However, Poverenov et al. (2014)

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found that single layer coating materials often cannot satisfy the diverse practical requirements such as effective barrier systems thereby calls for greater attention to bilayer or multi-component coatings. One of approaches for the bilayer coating applications is the layer-by-layer (LbL) electrostatic deposition. Poverenov et al. (2014) reported that the use of marine polysaccharides alginate and chitosan applied as edible coating in fresh-cut fruits such as melon could reduce problems during storage, for instance, weight loss, tissue softening, and vapor migration.

To the best of our knowledge, there have been no reports on the efficacy and barrier properties of the LbL electrostatic deposition technique for the development of edible coating for fresh-cut vegetables, such as lotus root. This is in contrast with the previous studies which were mainly focused on fresh-cut fruits (Poverenov et al. 2014; Souza et al. 2014) wherein problems such as tissue softening and weight loss occur during storage. Overall, the goal of this study was to develop a more effective edible coating system through the alternate deposition of opposite charged polyelectrolytes on the surface of fresh-cut lotus root using gum polysaccharide sources.

Materials and Methods

Chemicals

Xanthan gum (purity of < 95%) was purchased from MP Biomedicals (Tokyo, Japan) while guar gum, chitosan (80% deacetylation rate) were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan).

Plant Material and Processing Conditions

We prepared six experimental setups consisting of no coating (control), xanthan gum-coated only, guar gum-coated only, chitosan-coated only, xanthan gum + chitosan-coated, and guar gum + chitosan-coated. The control samples were freshly cut lotus root without any treatment. The three single coating treatments were separately applied as single layer to the fresh-cut lotus root slices while the two combined treatments (xanthan gum + chitosan-coated or guar gum + chitosan-coated) were applied as bilayers via the LbL electrostatic deposition of charges.

The lotus root (*N. nucifera*) samples used in this study were purchased from a local supermarket (Tsukuba, Japan). The samples were selected for freshness and good quality based on their whiteness and length of duration in the supermarket shelf as noted on the product labels. The samples were washed thoroughly in tap water, peeled, and sliced manually into 5 mm of thickness.

Preparation of the Polysaccharide Coating Solutions

In this study, 1 g of xanthan gum or guar gum was dissolved separately in 99 mL ultrapure, while 1 g of chitosan in 99 mL of 1% (w/w) acetic acid solution (pH = 2.44) to achieve 1% w/w concentration of edible coating solutions. These were magnetically stirred for 2 h at room temperature (HS 360 H, As One Corporation, Tokyo, Japan), for complete dissolution of the powder. Glycerol at 1% (w/w) was added as plasticizer for the edible coatings.

For the LbL deposition of electrostatic charges, the opposite charges of the polysaccharide (negatively charged xanthan gum and positively charged chitosan) were confirmed by dynamic light scattering, as indicated by the zeta potential values. For single layer coatings of xanthan gum, guar gum, and chitosan, the fresh-cut lotus root samples were immersed in the prepared coatings for 2 min and excess solutions of the coatings that had not adhered to the surface of the samples were let to drip. The samples were left to dry for 1 min at room temperature prior to packaging. Meanwhile, for the preparation of the samples treated with the bilayers of xanthan gum + chitosan or guar gum + chitosan, the fresh-cut lotus roots were firstly immersed in the 1st coating solution either xanthan gum or guar gum for 2 min. The excess coating solutions were let to drip off from the surface of the fresh-cut lotus root samples and air-dried for 1 min at room temperature. The fresh-cut lotus root slices were normally packed in polyethylene bags (60 μm × 160 mm × 260 mm; GT-1626, Kurilon, Sigma Tube, Tokyo, Japan) and were stored for 16 days at 5 °C for color, texture, enzymatic activity, total phenolic content, and morphological analyses.

Evaluation of Physicochemical Changes During Storage

Color

Color changes during 16 days of storage were monitored using spectrophotometer (CM-5, Konica Minolta, Tokyo, Japan). The L^* values were used to express lightness or darkness, a^* for redness and b^* for yellowness of the coated and non-coated fresh-cut lotus root samples.

Weight Loss During Storage

The weight loss from samples was also monitored during the 16 days of storage. The values were determined and expressed as percent losses from the initial weights of the sample using Eq. 1, as follows:

$$\%WL = \frac{w_0 - w_d}{w_0} \times 100 \quad (1)$$

where w_0 is the initial weight at day 0 (g), and w_d is the final weight at each storage day interval (g).

Statistical Analyses

All experiments were performed in at least triplicates and the results were reported as the average ± standard deviation of the measurements. The SPSS Statistics (IBM Statistics 22, New York, USA) was used to apply one-way analysis of variance (ANOVA) to analyze the data for color and weight loss. The Duncan multiple range test was used as post hoc test at a 95% confidence level. The significant difference among the treatments ($p < 0.05$) was indicated using different letters.

Results and Discussion

Changes in Color of Coated Lotus Root Samples upon Storage

Color is one of the most important quality attributes that a consumer easily considers for selection and purchase. Figure 1 a shows the effect of the edible coatings on the whiteness (L^* values). The L^* value was used to indicate the lightness of the samples. Higher whiteness (L^* value) indicates brighter surface of samples (Du et al. 2009). During storage, the whiteness values tend to decrease, due to the naturally occurring enzymatic browning of fresh-cut lotus root. Our results have shown that after 4 days of storage at 5 °C, the L^* values of control samples had decreased significantly ($p < 0.05$) and among the treatments had the lowest whiteness values, which imply that enzymatic browning occurred during storage. These results also indicate that control samples oxidize easily during storage. Meanwhile, L^* values of samples treated with edible coatings were significantly higher ($p < 0.05$) than those of the control samples. Among these edible coatings, the bilayer coatings of xanthan gum + chitosan (XG + Ch) resulted in highest whiteness values. No significant whiteness change ($p > 0.05$) was observed for XG + Ch samples stored from day 0 to 4. The electrostatic interaction between the positively charged chitosan and the negatively charged xanthan gum could probably have improved the barrier properties of the XG + Ch coatings applied to the fresh-cut lotus root. According to previous reports, the interaction of these two polymers in aqueous solutions forms a hydrogel network. The electrostatic interaction of the polymers which occurs between the positively charged amino groups ($-NH_3^+$) of chitosan and the negatively charged carboxyl groups ($-COO^-$) in xanthan gum creates strong hydrogels (Dadou et al. 2017). From storage days 4 to 16, whiteness values (L^*) of control samples continued to decrease, implying further oxidation of the samples. This can be attributed to the absence of a protective layer, like that of the edible coatings,

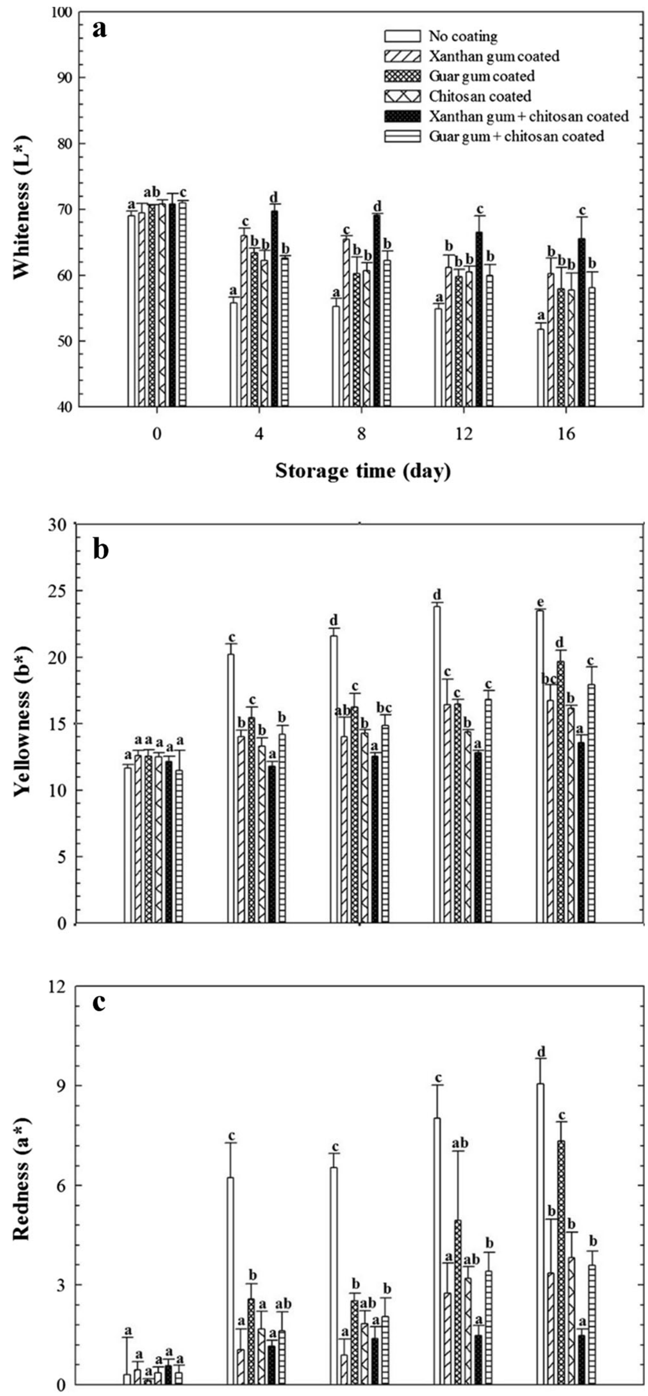

Fig. 1 a–c Color changes of fresh-cut lotus root. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$)

which serves as a barrier against oxidation. Fresh-cut lotus root samples treated with xanthan gum, guar gum, and chitosan exhibited higher whiteness values (L^*) than control samples during storage of 16 days at 5 °C. XG + Ch-treated samples exhibited the highest whiteness values (L^*) after 16 days of storage. Zhou et al. (2018) obtained the similar effect of konjac glucomannan coating on maintaining the L^* values of fresh-cut lotus root during storage; however, after 8 days, L^* values of coated samples started to decrease.

Meanwhile, the increasing a^* and b^* values of fresh-cut lotus root indicated aggravating red and yellow color, respectively in the fresh-cut lotus root samples which is in agreement with previous literature Xing et al. (2012). As observed in Fig. 1 b and c, the control lotus root samples had the fastest increase of a^* and b^* values during storage for 16 days while the slowest increase was observed for XG + Ch-treated samples.

Changes in Weight Loss of Coated Lotus Root Samples upon Storage

Weight loss is one of the limiting factors for shelf-life of fresh-cut vegetable that results to degradation of appearance, texture, and nutritional qualities (Freitas et al. 2013). Excessive weight loss makes food products unsuitable for consumption and marketing. Increasing weight loss of fresh-cut lotus root samples was observed during 16 days of storage. However, it was found that after 4 days, weight loss of all coated samples was significantly lower compared with the untreated sample (Fig. 2). Among these coatings, the single layer chitosan coating and LbL coatings of xanthan gum + chitosan and guar gum + chitosan were the most effective in decreasing weight loss in fresh-cut lotus root samples. The same trend was observed in samples stored up to 8 and 16 days. The use of LbL coating of xanthan gum + chitosan resulted in the lowest weight loss of the sample. The decreased weight loss among the fresh-cut lotus root samples during storage can be attributed to the application of edible coatings, which acts as a semipermeable barrier against oxygen, carbon dioxide, and moisture, thus, reducing respiration, water loss, and oxidation reactions (Ali et al. 2010). Meanwhile, the increased barrier properties of xanthan gum + chitosan may be due to the electrostatic interactions between the two opposite charged polysaccharides, which leads to the increase in matrix tortuosity thus reducing the migration and movement of molecules through the matrix materials (Souza et al. 2014). Experimental results of Carneiro-da-Cunha et al. (2010) also showed that the electrostatic interaction of alginate-chitosan nanolayers increased the barrier properties of the films against water vapor loss.

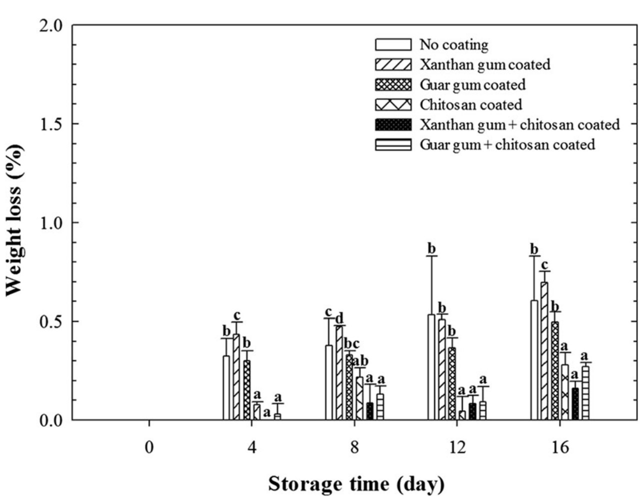

Fig. 2 Weight loss changes of fresh-cut lotus root during storage at 5 °C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$)

Conclusions

In our study, we highlighted the improved performance of the LbL electrostatic deposition technique in the application of edible coatings to fresh-cut lotus root than to single layer alone. Based on our results, the LbL coatings of xanthan gum and chitosan showed higher functionality than the single layer polysaccharide-based coatings thus increasing its barrier properties against oxidation and water vapor loss. In addition, the improved performance of the LbL edible coatings was achieved without the further addition of antioxidants and antimicrobial agents. Moreover, this method is a simple and cost-effective technique and is a promising strategy in increasing the storage stability of fresh-cut lotus root especially during transport and distribution.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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