Development of Edible Coatings for Enhancing the Storage Stability of Fresh-Cut Lotus Root (*Nelumbo nucifera*)

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Development of Edible Coatings for Enhancing the Storage Stability of Fresh-Cut Lotus Root (*Nelumbo nucifera*)

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List of abbreviations

GG	Guar gum
XG	Xanthan gum
LbL	Layer-by-layer
PPO	Polyphenol oxidase
CA	Citric acid
POD	Peroxidase
GAE	Gallic acid equivalents
SEM	Scanning electron microscope

Abstract

Minimal processed fruits and vegetables such as fresh-cut products have been gaining popularity among consumers because of convenience and freshness. An example of a fresh-cut product gaining interest from researchers and industries is the fresh-cut lotus root (*Nelumbo nucifera*). Its white color, crisp texture, pleasant aroma, and high nutritional content make this rhizome appealing to consumers. However, fresh-cut lotus root is prone to enzymatic browning and as a result, deteriorates its quality and shortens its shelf-life. Various strategies. One of the alternative and promising approaches for the shelf life extension of fresh-cut lotus root is the application of edible coatings om researchers have been employed to improve the shelf-life of fresh-cut lotus root (Chapter 1).

In Chapter 2, the author targeted the formulation of polysaccharide-based edible coatings with different ionic charges from natural gum sources, such as guar gum, xanthan gum, and chitosan. The results have shown that LbL 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. Decreased polyphenol oxidase (PPO) enzymatic activity up to 70% was also observed in coated samples as compared to the non-coated samples. The author also found that morphological analyses proved that edible coatings have maintained cell wall integrity of samples during storage.

Previously, the author reported in Chapter 2 that xanthan gum-chitosan edible coatings applied through the LbL electrostatic deposition of charges had the highest

barrier properties in preventing oxidation during storage of fresh-cut lotus root. The author, then, evaluated the influence of xanthan gum concentration in improving the barrier properties of xanthan gum-chitosan edible coatings on fresh-cut lotus root in Chapter 3. The results have shown that layer-by-layer coatings of 1.5% xanthan gum and chitosan were the most effective in decreasing the total color changes and enzymatic activity of fresh-cut lotus root during storage for 16 days at 5°C. In this part of the research work, the author concluded that increasing the xanthan gum concentration in xanthan gum-chitosan edible coatings resulted to stronger barrier against oxidation and enzyme activity in fresh-cut lotus root.

In Chapter 2 and 3, the author developed polysaccharide-based edible coatings with different ionic charges from gum sources, such as guar gum, xanthan gum and chitosan and applied to the fresh-cut lotus root through dipping. However, due to the limitations of the dipping process such as uneven thickness of coating layer, the author had been interested in an alternative application technique, which is the spraying method. In Chapter 4, the author evaluated the effect of the spraying method as an application technique for xanthan gum-based edible coatings and investigated its barrier and microbial properties on fresh-cut lotus root.

It was found that spray-coated fresh-cut lotus root samples had significant reduction in the total color changes as compared to non-coated samples. These results suggest that the spray coating treatments were effective in decreasing the enzymatic browning of fresh-cut lotus root during storage which could potentially increase its shelf-life in the market. In addition, the author also found that the xanthan gum-based spray coated treatments were also effective against inhibiting the growth of *Bacillus subtilis*

during 24 h of incubation which were indicated by the lower microbial counts recorded as compared to non-coated fresh-cut lotus root samples. In this part of the work, the author highlighted the spray coating technique of xanthan gum-based edible coatings as a promising strategy in improving the storage stability of fresh-cut lotus root during post-harvest storage.

Dipping and spraying methods of coating applications were also investigated in this work and storage stability parameters were also evaluated. However, based on the obtained results, no significant difference was found between the dipping and spraying method, in terms of the reduction of color changes during storage for 16 days at 5°C. Overall, the application of edible coatings is a promising strategy in extending the shelf life of fresh-cut lotus root. In the future, the author aims to widen the scope of the application of these coatings to other agricultural products which are prone to degradation during storage in the market.

Chapter 1

General Introduction

1.1. Fresh-cut products

It has been reported that there is an expansion of fresh-cut fruits and vegetable market over the years (Qadri et al., 2015). This recent growth is attributed to the consumer demand for healthy, fresh, convenient, and additive-free foods (Gorni et al., 2015; Francis et al., 2012).

But, fresh-cut products are prone to perishability and shorter shelf life, compared to intact products because of severe physical stress these have undergone, such as peeling, cutting, slicing, shredding, trimming and/or coring and removal of protective epidermal cells (Watada et al., 1996).

According to Soliva-Fortuny et al. (2003), minimally processed products are considered wounded tissues. The degree of wound response is influence by several factors such as species and variety, O_2 and CO_2 concentrations, water vapor pressure, and the presence of inhibitors. Due to this wounding, several undesirable changes occur such as excessive tissue softening, water loss, surface browning, nutrient loss, off-flavor production, and microbial spoilage. Additionally, the growth of pathogenic microorganisms is also promoted due to the exposed area after minimal processing. Foodborne disease outbreaks from the consumption of fresh-cut products have been reported and led to serious concerns towards the public (Ma, et al., 2017).

The development of preservation techniques for fresh-cut products has been a major concern to address their issues of limited shelf-life. Ma et al. (2017) reported three classifications of preservation techniques: (1) physical-based preservation ; (2) chemical-based preservation; and (3) biopreservation technology. Physical methods are referred to techniques that vary environmental temperature, humidity, pressure, and gas composition. Examples of these are modified atmosphere packaging (MAP) and cold storage. Chemical

methods, on the other hand, consist of the use of natural or synthetic preservatives to extend the shelf life of fresh-cut products. Garcia & Barrett (2002) reported dipping treatments from acids (citric acid, ascorbic acid, acetic acid, and malic acid) and calcium salts. Finally, the biopreservation technology utilizes the antimicrobial potential of natural microorganisms and their corresponding antibacterial products to extend shelf life and improve the safety of food.

1.2. Edible coatings

Edible coatings are defined as a thin layer of material which cover a food product and extend its shelf life (Raghav et al., 2016). Edible coatings are applied to fresh-cut fruits and vegetables to provide protection, enhance appearance, reduce water vapor and air transfer between food product and environment, and decrease respiration rates and enzymatic activities (Lin & Zhao, 2007).

Edible coatings can be sourced out from different sources such as polysaccharides, proteins, and lipids. Polysaccharides are good materials for edible coatings due to their excellent mechanical and structural properties; however, have weak poor ability against moisture transfer. Sources of polysaccharides include starch and derivatives, chitosan, pectin, cellulose and derivatives, alginate, and carrageenan (Lazi et al., 2015). Proteins are also another material for edible coating which have excellent gas and lipid barrier properties. Examples of these are casein, gelatin, collagen, and gluten. In addition, lipids are also sources of edible coatings which possess excellent gas and water barrier properties. These type of coatings can be sourced out from waxes, fatty acids, and shellac resins (Yai, 2008).

Additives such as antioxidants, acidulants, fungicides, preservatives, and plasticizers are incorporated to improve their physical, mechanical, and barrier properties (Baldwin et al., 1996). Plasticizers are one of the ingredients added to coatings to improve their mechanical quality. McHugh & Krochta (1994) defined plasticizers as "a substantially nonvolatile, high boiling, nonseparating substance, which when added to another material changes the physical and or/ mechanical properties of that material". Plasticizers such as glycerol and sorbitol reduce internal hydrogen bonding and increase intermolecular spacing, which contribute to their ability to effectively plasticize coatings.

Plasticizers are speculated to reduce the intermolecular forces along polymers chains; hence, increased film flexibility but reduced barrier properties. Debeaufort & Voilley (1997) reported that the use of polyethylene glycol (PEG) in methylcellulose-based films increased intermolecular spacing within the molecules and reduced their interactions, and thus, decreased their tensile strength.

1.3. Xanthan gum

Xanthan gum, as described, is a polysaccharide produced from synthesis of carbon sources from *Xanthomonas* genus (Freitas et al., 2013). It is a high-molecular weight polysaccharide which is composed of D-glucose, D-mannose, and D-glucuronic acid (Merlusca et al., 2016). The presence of carboxylic groups, as shown in Fig. 1.1, makes xanthan gam a weak acid which acts as an anionic polyelectrolyte in pH conditions higher than 3.1. Due to these negative charges, xanthan gum has the ability to form complexes with positively charged polymers (Dadou et al., 2017).

Xanthan gum is a Generally Regarded as Safe (GRAS) compound, which has been widely utilized as stabilizer, thickener, and emulsifier in food applications. This polymer forms viscous solutions in cold or hot water at low concentration with a good stability over a wide range of pH and temperature, and is also resistant to enzymatic degradation (Sharma & Rao, 2015). Moreover, xanthan gum has also been applied as edible coatings to improve the surface color of baby carrots and easy peelers and to reduce the supply of oxygen for the shelf life extension of prickly pear (Quoc et al., 2016).

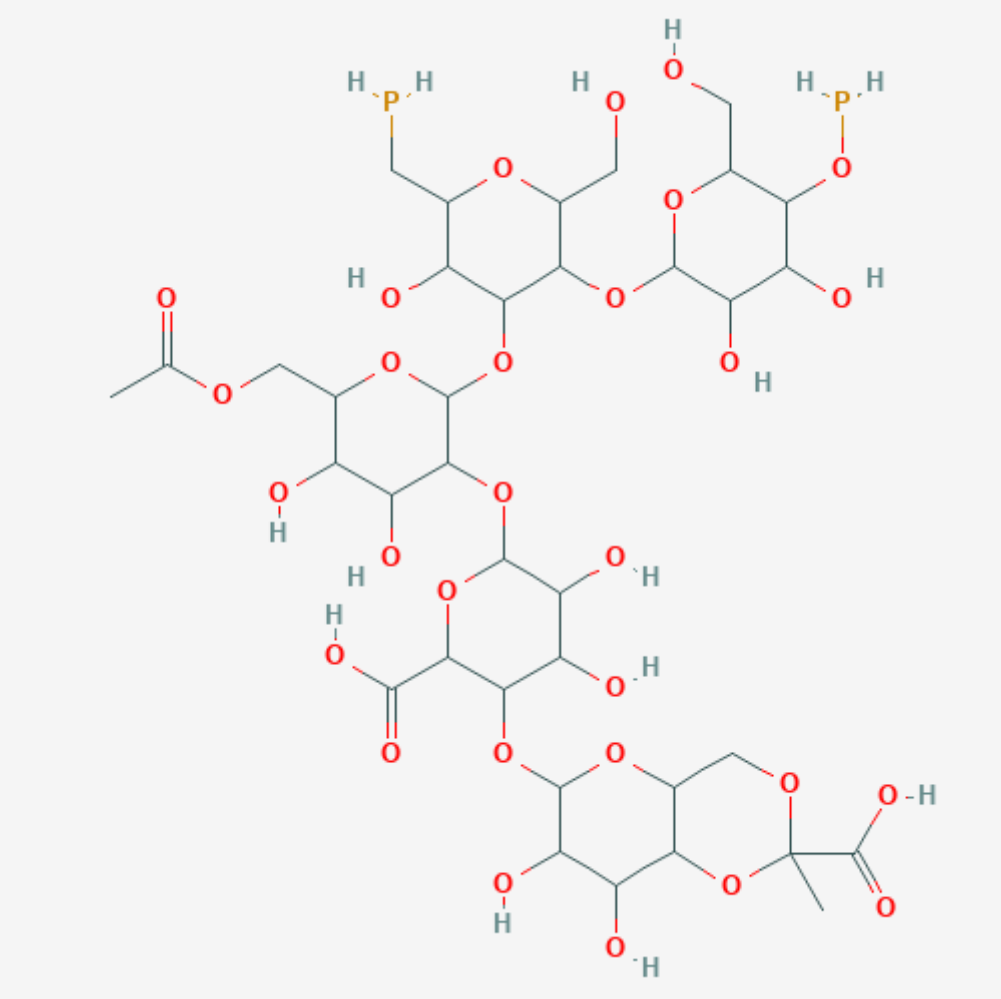


Fig. 1.1 Chemical structure of xanthan gum

(Source: https://pubchem.ncbi.nlm.nih.gov/compound/XC-Polymer#section=2D-Structure)

1.4. Guar gum

Guar gum is a polysaccharide extracted from the seeds of *Cyamopsis tetragonoloba* and is commercially grown in Asia, North Africa, and South America (Mudgil et al., 2014; Gong et al., 2012). It has a backbone composed of linear chain (1-4) β-D-mannopyranose with galactose as a side group linked to (1-6) α-D-galactopyranose (Saberi et al., 2016). It has an excellent hydration property due to its hydroxyl bonds, shown in Fig. 1.2. Guar gum forms hydrogen bond with cellulosic materials and hydrated minerals. Due to its nonionic properties, guar gum is stable and gives a consistent viscosity over a wide range of pH (Thombare et al., 2016).

Due to low cost and ability to form viscous solutions at low concentrations, guar gum has been used in several industrial and food applications such as emulsifier, thickeners, or stabilizers for wide range of processed foods. The high viscosity of guar gum solutions is due to the high molecular weight of this polysaccharide and the presence of extensive intermolecular entanglement through hydrogen bonding (Gong et al., 2012). Thombare et al. (2016) stated that the guar gum exhibit a shear thinning property with non-Newtonian pseudoplastic flow. This implies that as shear rate increases, the viscosity of guar gum also decreases.

Mudgil et al. (2014) reported that guar gum is utilized as a food additive to emulsify, bind water, prevent ice crystals in frozen products, moisturizes, thickens, stabilizes, and suspends many liquid-solid systems. It has been applied to ice cream, sauces, cake mixes, cheese spreads, fruit drinks, and dressings.

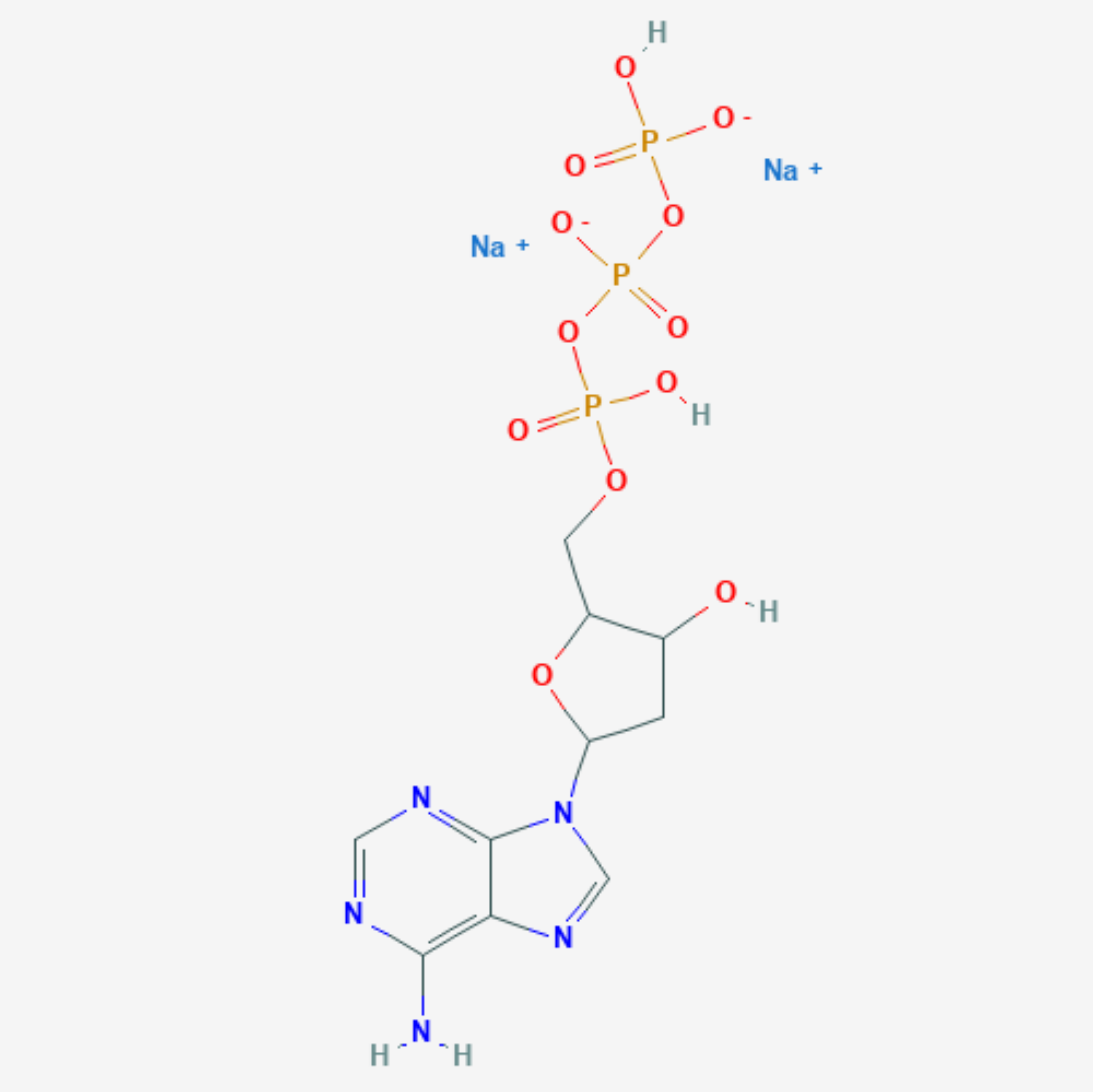


Fig. 1.2 Chemical structure of guar gum

(Source: https://pubchem.ncbi.nlm.nih.gov/compound/Guar-gum#section=2D-Structure)

1.5. Chitosan

Chitosan, as shown in Fig. 1.3, is a high molecular weight cationic linear polysaccharide. It is a deacylated derivative of chitin, which is usually extracted from shellfish exoskeleton sources or microorganism and fungi cell wall (Petriccione et al., 2015). Chitosan is considered to be a co-polymer of N-acetyl-glucosamine and N-glucosamine units which are randomly or block distributed throughout the polymer chain (Khor & Lim, 2003). This polysaccharide is a highly insoluble material, which is similar to cellulose, in terms of solubility and low chemical reactivity (Kumar, 2000). In addition, two of the most significant properties of chitosan include its cationic or positive charge and high charge density in acidic solution which enables chitosan to form insoluble ionic complexes with water-soluble polyanionic species in neutral conditions (Vasiliu et al., 2005) .

Chitosan, known as a polyelectrolyte, could form electrostatic complexes in acidic conditions with an oppositely charged surfactant (SPEC) and polyelectrolyte complexes (PEC). Reported polymers with electrostatic interaction with chitosan include polyacrylic acid, sodium salt (PAA), carboxymethylcellulose (CMC), xanthan, carrageenan, alginate (from brown algae), pectin, dextran, and sulfate (Rinaudo, 2006). Electrostatic complexes of chitosan with other polymers could be applied as antithrombogenic materials, controlled release systems, encapsulation of drugs, immobilization of enzymes and cells, and gene carriers (Vasiliu et al., 2005).

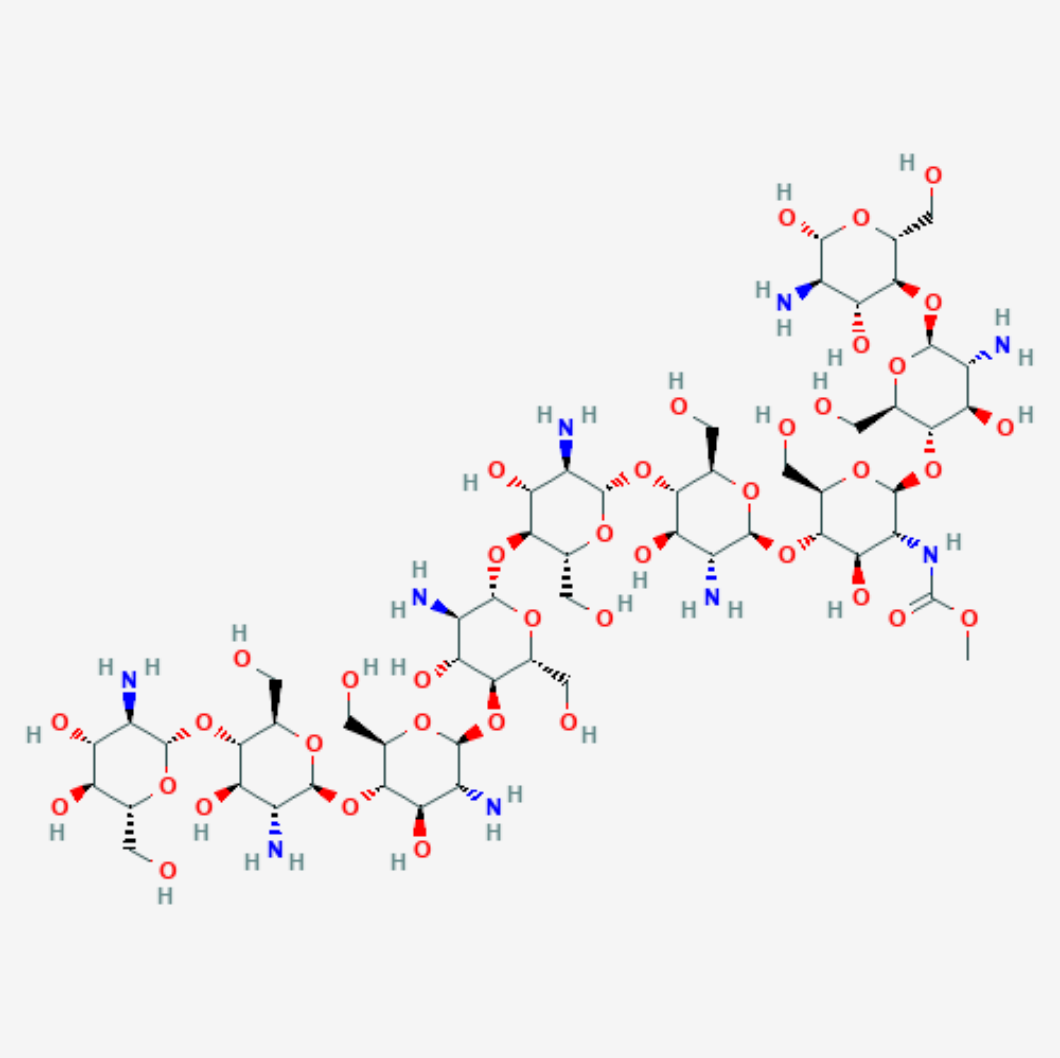


Fig. 1.3 Chemical structure of chitosan

(Source:https://pubchem.ncbi.nlm.nih.gov/compound/Chitosan#section=Structures)

1.6. Edible coating applications

Edible coatings could be applied through panning, fluidized bed, dipping, and spraying. In the panning process, food products are coated in a large, rotating bowl, known as “pan”. The coating solution is applied to the product by spraying and tumbling the pan to evenly distribute the coating material (Andrade et al., 2012). Meanwhile, during the fluidized bed coating process, coating materials are sprayed through the food product using a set of nozzles onto the surface of fluidized powders to form a shell type structure. Fluidized bed are classified according to three configurations: top spray, bottom spray, and rotating fluidized bed (Dewettinck & Huyghebaert, 1999). The dipping process, on the other hand, is the most common laboratory scale technique because it is a simple, cheap, and efficient process (Zhong et al., 2014). The dipping process involves immerging the food product in a container carrying the coating solution. Finally, in the spraying process, coating materials are applied to food products using high pressure applicators and air atomizing systems (Silva-Vera et al., 2018). During the process, it was reported that the surface area of the liquid is increased through the formation of droplets, which are then distributed to the food surface through a set of nozzles (Andrade et al., 2012).

1.7. Lotus root (Nelumbo nucifera)

The lotus root (*Nelumbo nucifera*), as shown in Fig. 1.4, is a vegetable cultivated in various countries such as Japan, Korea, China and Vietnam (Tu et al., 2015).

Fig. 1.4 Lotus root sold in Japan

In Japan, most of the lotus root production originates from the Ibaraki region. The actual lotus root production in Tsuchiura, Ibaraki region is presented in Fig. 1.5. According to the Ministry of Agriculture, Forestry and Fisheries of Japan, the Ibaraki prefecture has a yearly total production of 30,500 tons, which accounts for 49% of the total production of the country (Fig. 1.6).

Fig. 1.5 Actual lotus root production in Tsuchiura city, Ibaraki Prefecture, Japan

Park et al. (2009) reported that lotus cultivars consist of high nutritional content which could make the plant a potential medicinal food. Results have shown that the lotus root contains 13% protein, 2.3 % crude fat, 66 % carbohydrates and 2.8 % fiber. On the other hand, potassium levels were reported about 30%, calcium was 27 %, phosphorus was 4.4 % and magnesium amounts were 28.7%. There are also high levels of ascorbic acid which make lotus root higher in nutrition. Studies of Tsuruta et al. (2011) proved that polyphenolic extracts of lotus root resulted to suppression of hepatic lipogenesis by the lotus root polyphenols.

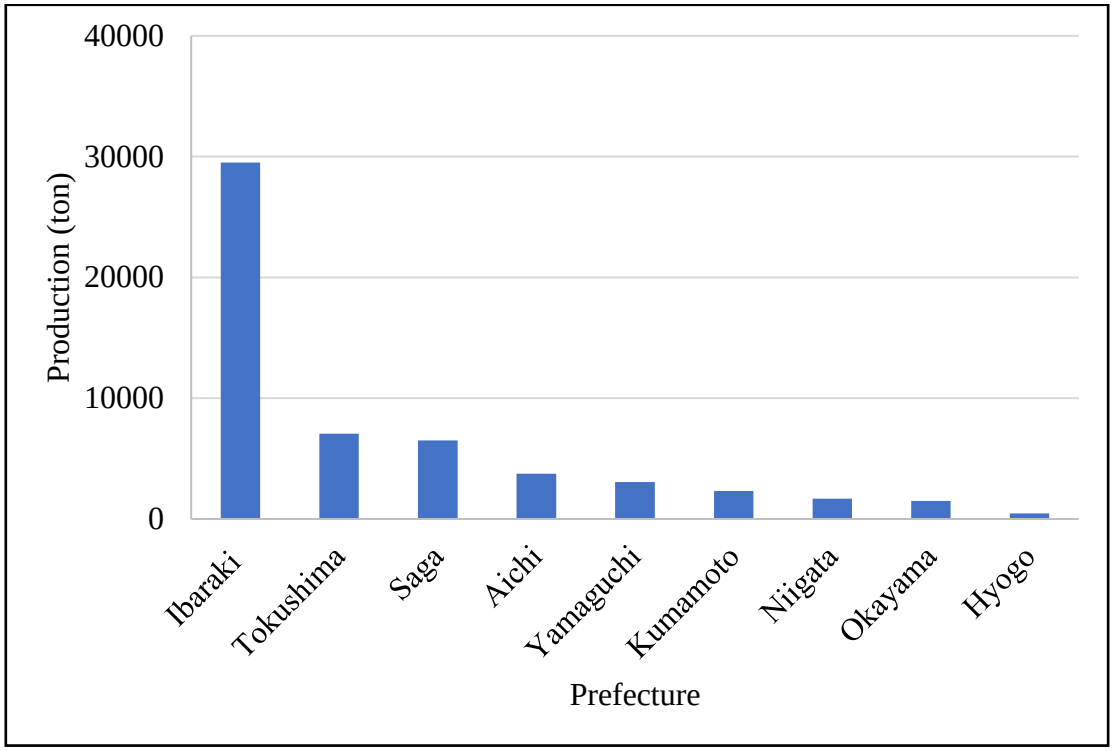


Fig. 1.6 Lotus root production per Japan prefecture.

(Ministry of Agriculture, Forestry and Fisheries of Japan, 2017)

1.8. Objectives of this research work

The general objective of this research is to develop edible coatings from xanthan gum, guar gum, and chitosan. In addition, the study was specifically aimed to:

(1) Evaluate the effect of edible coatings, applied via single and bilayers, on quality changes of fresh-cut lotus root such as color, texture, enzymatic activity, total phenols, and microstructural changes during 16 days of storage

(2) Determine the influence of xanthan gum concentration on improving the barrier properties of xanthan gum – chitosan edible coatings on fresh-cut lotus root

(3) Investigate the spraying method as an alternative technique for the application of edible coatings on fresh-cut lotus root

Chapter 2

Layer-by-Layer Electrostatic Deposition of Edible Coatings

2.1. Introduction

The demand for fresh-cut fruits and vegetables in the market has been increasing through the years because of convenience and freshness that these products offer to consumer's busier lifestyles (Qadri et al., 2015) . However, due to physical damage during processing such as peeling, coring, cutting, slicing, and trimming, fresh-cut products are more susceptible to spoilage, thus reduced shelf life as compared to intact products (Watada et al., 1996)

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 are 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 et al. (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. Xing et al. (2012) studied on the combined effects of anti-browning agents, cinnamon oil fumigation and moderate vacuum packaging. Zhang et al. (2013) evaluated carbon monoxide on browning of fresh-cut lotus root while Xing et al. (2010) evaluated the combined effects of chitosan-based coating and modified atmosphere packaging

(MAP). Therefore, the author 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.

The application of edible coatings has been found to be a promising strategy in protecting food products from mechanical, physical, chemical, and microbial damages so that extending their shelf life (Poverenov et al., 2014) . The importance of edible coatings has been increasing in the food sector because these can be a substitute treatment in reducing the deterioration caused by minimal processing in fruits and vegetables, due to the semi-permeable barrier to gases and water vapor which these edible coatings can provide. As a result, respiration rates, enzymatic browning and water loss maybe reduced (Sanchís et al., 2017).

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, were found to inhibit peroxidase (POD) enzyme activity and slowed the decrease of total phenolic and flavonoid contents in pears (Kou et al., 2014); the application of aloe vera and gum taracanth in button mushrooms delayed weight loss, color changes and softening in button mushrooms (Mohebbi et al., 2012) and moreover, the use of tocopherol nanocapsules/xanthan gum coatings decreased respiration and oxidation rates in fresh-cut apples during 21 days of storage (Galindo-Pérez et al., 2015). However, Poverenov et al. (2014) 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 multicomponent 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. In a related study, it was also accounted that both blended and LbL electrostatically deposited edible coatings of gelatin and chitosan on fresh-cut melon model were effective in maintaining fruit texture, reducing fruit weight loss and inhibiting microbial growth during storage (Poverenov et al., 2014). Other previous works on the LbL coatings such as from chitosan and carboxymethylcellulose (CMC) had shown that these coatings maintained the firmness and characteristic volatiles of strawberries during storage (Yan, Luo, Ban, Lu, Li, Yang, et al., 2019) while the LbL alginate and chitosan edible coatings enhanced the microbiological and physico-chemical properties of fresh-cut mangoes during storage (Souza et al., 2014).

To the best of the author's 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. In addition, our work also emphasized the significance of the LbL electrostatic deposition of opposite charged materials - xanthan gum and chitosan as an effective barrier against oxidation and enzymatic browning, which are predominant in fresh-cut lotus root products. The author have also incorporated new insights in our work by providing morphological analyses and wetting properties (contact angle measurements) of these edible coatings on the fresh-cut lotus root surface to further

elaborate the possible mechanism of the improved barrier properties of the resulting edible coatings on the fresh-cut lotus root. 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. To the best of the author's knowledge, xanthan gum and chitosan combination has not been applied as an LbL electrostatically deposited edible coatings for any fresh-cut product, such as vegetable or fruits. In this work, the authors firstly evaluated the effect of plasticizers on the rheological properties of edible coatings from xanthan gum, guar gum or chitosan. Subsequently, the resulting coatings were characterized and applied through single or bilayers and assessed its effect on the physico-chemical and morphological aspects of the fresh-cut lotus root.

2.2. Materials and methods

2.2.1. Chemicals

Xanthan gum (purity of <95%) was purchased from MP Biomedicals (Tokyo, Japan) while guar gum, chitosan (80% deacetylation rate), polyvinylpyrrolidone, catechol, sodium phosphate, Folin-Ciocalteu reagent, and gallic acid (purity of 100%) were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan).

2.2.2. Plant material and processing conditions

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 were prepared. 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 (Fig. 2.1).

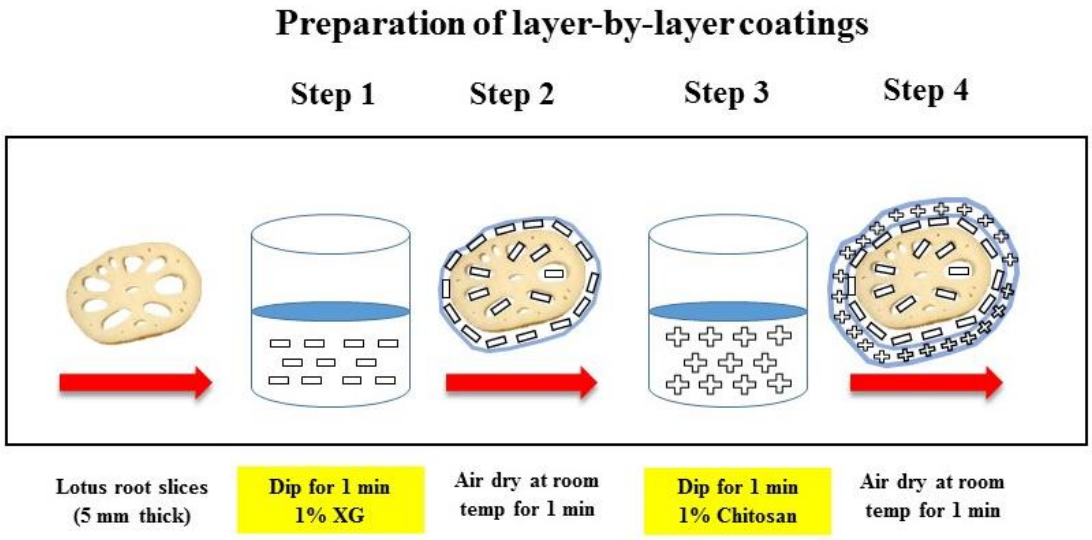


Fig. 2.1 Schematic presentation of the electrostatic deposition applied as layer-by-layer edible coatings to fresh-cut lotus root. (XG: Xanthan gum; GG: Guar gum)

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. The edible coatings were applied on the fresh-cut lotus root via the immersion technique. After the application of the coatings, 10 slices of fresh-cut lotus root were packed per treatment in polyethylene bags (60 μm x 160 mm x 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.

2.2.3. Preparation of the polysaccharide coating solutions

In this study, 1 g of xanthan gum or guar gum were 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. Preliminary experiments were performed to determine the appropriate plasticizer for the edible coatings. The effect of glycerol and oleic acid as a plasticizer on the physical and rheological properties of the edible coatings were evaluated. Based on our results, we decided to use glycerol due its compatibility to the polysaccharide-based edible coatings. The influence of different concentrations (1, 2, and 3% w/w) of glycerol on the physical and rheological properties of the coating solutions was investigated. Analyses of pH, viscosity, and optical microscopy were performed to understand the effect of varying concentrations of glycerol on the solutions. Furthermore, the final

polysaccharide coatings that were applied to the fresh-cut lotus root were analyzed for viscosity, pH, and contact angle.

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 and were stored for 16 days at 5 °C for color, texture, enzymatic activity, total phenolic content, and morphological analyses.

2.2.4. Analysis of the polysaccharide coating solutions

2.2.4.1. Viscosity

The viscosity of the coating solutions from xanthan gum, guar gum, and chitosan were analyzed using a Vibro Viscometer (SV-10, A & D Company Ltd., Tokyo, Japan) at room temperature. 35 mL of the liquid sample was placed in the vessel for measurement.

2.2.4.2. Morphological analyses

Images of the polysaccharide-based coating solutions were analyzed using optical and fluorescence microscope (DM-IRM Leica Microsystems, Weizler, Germany). A drop from each coating solution was deposited on the slide glass and was covered with a cover slip prior to analysis. The images were magnified 5 times for analysis.

2.2.4.3 Zeta Potential

The confirmation of the charges of the polysaccharides for the LbL electrostatic deposition was confirmed through the measurement of the Zeta-potential (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK). The samples were loaded in a folded capillary cell and were then automatically measured using the instrument.

2.2.4.4. pH

The pH of the coating solutions from xanthan gum, guar gum, and chitosan were determined using a digital pH meter (827 pH lab, Metrohm, Herisau, Switzerland). 50 mL of the liquid sample was placed in the vessel for measurement.

2.2.4.5. Coating formation

The coating formation of the polysaccharide-based coating solutions were investigated through the scanning electron microscope (SEM Miniscope 1000, Hitachi, Tokyo, Japan). Prior to microscopy, the coated and non-coated samples were frozen for 24 h and freeze-dried for 24-48 h (FDU-2100 Eyela Desktop Freeze Dryer, Tokyo, Japan). After freeze-drying, the samples were mounted on aluminum-made sample probes to record SEM images at 300x magnification.

2.2.5. Evaluation of physico-chemical changes during storage

2.2.5.1. Color

Color changes during 16 days of storage were monitored using spectrophotometer (CM-5, Konica Minolta, Tokyo, Japan). The 16 days storage time was decided to have enough time to observe the color, textural, enzymatic and microstructural changes and to determine the stage at which enzymatic browning take place after the application of the coating treatments. 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.

2.2.5.2. Texture

The textural changes were evaluated with the Texture Profile Unit (TPU 2DL, Yamaden, Tokyo, Japan). The 5-mm thick fresh-cut lotus root slices were compressed longitudinally with a probe at a deformation rate of 2.5 mm/s. The hardness value, which is defined as the peak force at the first compression cycle, was expressed in Newton (N).

2.2.5.3. 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. 2.1, as follows:

$$\% WL = \frac{w_0 - w_d}{w_0} \times 100 \qquad (2.1)$$

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

2.2.5.4. Polyphenol oxidase activity (PPO)

The method for the determination of the enzymatic activity of polyphenol oxidase (PPO) was based on the standard procedures reported by Son et al. (2015) with few modifications. Ten grams of each fresh-cut lotus root sample per treatment were manually homogenized using mortar and pestle with sodium phosphate buffer (0.2 mol/L, pH 7.0 with 2% polyvinylpyrrolidone) following a ratio of 2.0 mL of buffer per 1 g of lotus root in an ice bath. Centrifugation (high-speed refrigerated centrifuge, Tomy MX -307, Tomy Seiko, Tokyo, Japan) of the homogenates was done at 12000 × g for 10 min at 4 °C. The supernatant was separated from the filtrate and was further used for the analysis. The enzymatic activity was evaluated by the increase of absorbance at 410 nm for catechol at 25 °C using a spectrophotometer (UV-Vis, V-530, JASCO Inc., Tokyo, Japan). The 0.2 mL supernatant was added with 2.8 mL of the catechol substrate solution (0.02 mol/L catechol in 0.05 mol/L sodium phosphate buffer, pH 7). As reference, the catechol substrate solution (catechol in sodium phosphate buffer) was used. The enzymatic activity (units/ (min-mL) enzyme)) was determined by the linear section of the activity curve. The

change in 0.001 in the absorbance value per min was defined as 1 unit of PPO enzyme activity.

2.2.5.5. Total phenolic content

The method used to evaluate the total phenolic content of the fresh-cut samples was based on procedures reported by Xing et al. (2012) with a few modifications. Ten grams of lotus root sample per treatment were crushed with 25 mL ultrapure water using a mortar and pestle. Centrifugation was performed for 15 min at 5000 × *g*. One milliliter of the supernatant was mixed with 5 mL Folin-Ciocalteu solution (1 mL of FC reagent in 10 mL ultrapure water). Between 0 and 8 min of reaction time, 4 mL of 7.5% w/v sodium carbonate solution were slowly added. Samples were incubated for 2 h at 30 °C in a controlled incubator (Panasonic cool incubator, ICI-200, Tokyo, Japan). A spectrophotometer (UV-Vis, V-530, JASCO Inc.) was used to measure the absorbance of the solution at 760 nm. Gallic acid was used as a standard for the analysis. Solutions of this acid at concentrations ranging from 0.01 to 0.1 mg/mL were prepared in ultrapure water to plot a calibration curve ($8.4219x + 0.0859$, $R^2=0.99$). The results were calculated from this curve and expressed as mg gallic acid equivalents (GAE) per gram of fresh-cut lotus root.

2.2.6. Evaluation of morphological changes during storage

Morphological analyses were performed using scanning electron microscope (SEM Miniscope 1000, Hitachi, Tokyo, Japan). Prior to microscopy, 2 g of samples per treatment were sliced to 1 mm^3 cubes and then oven dried at 100°C for 5 h to achieve 16% ± 2.0 total solids contents (w/w). After drying, samples were mounted on aluminum-

made sample probes to record SEM images at 500x magnification. Samples were observed up to 16 days storage at 5 °C storage.

2.2.7. 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 viscosity, pH, texture, color, weight loss, enzymatic activity and total phenolic content. 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.

2.3. Results and discussion

2.3.1. Effect of glycerol concentration on the edible coating properties

Plasticizer addition is essential in the formulation of edible coatings for improving coating integrity by preventing the development of pores and cracks (Rojas-Argudo et al., 2009). Glycerol, known for its gloss-enhancing ability, is a widely used plasticizer to enhance the mechanical properties of biopolymers (Arnon et al., 2015) . In this study, the effects of different concentrations of glycerol (1, 2, and 3% w/w basis) on edible coating viscosity and pH of xanthan gum, guar gum, and chitosan were determined as shown in Fig. 2.2a and Fig. 2b. Previous study of Sipahi et al. (2013) reported that the use of 2% glycerol resulted uniform and stable edible alginate based coating, however, due to the differences of polysaccharides such as alginates and gums, it was decided to evaluate the effect of glycerol contents below and above 2% (w/w) concentrations on the rheological properties of xanthan gum, guar gum, and chitosan edible coatings.

For xanthan gum, guar gum, and chitosan coating solutions, we found out that the addition of 1% (w/w) glycerol, initially increased the viscosities of the coatings (Fig. 2.2a). This may be due to possible formation of a stronger network of glycerol and polysaccharides at low concentrations of 1% glycerol. However, after increasing the concentration of glycerol in the coating solutions to 2% and 3%, we observed significantly reduced viscosities by 30% and 15% for guar gum and xanthan gum, respectively. It is possible that at higher concentrations of the plasticizer, the mobility of the polymer chains were increased and the stiffness of the networks were reduced, thus resulted to lower viscosity values (Navarro-Tarazaga et al., 2008; Saberi et al., 2016).

In this study, the author evaluated the effect of varying concentrations of glycerol on the pH of the edible coatings (Fig. 2.2b). Evaluating the effect on pH is important because the charges of the different polysaccharides used in this work were dependent on pH alterations. The charges of the polysaccharides, as measured by the zeta-potential values, were as observed: 1% xanthan gum aqueous solution has negative charge (-67.33 mV), 1% chitosan aqueous solution has positive charge (+68.37 mV) while 1% guar gum aqueous solution has a neutral charge.

For chitosan, its charged properties are affected by the presence of the amine groups. At pH lower than 6.5, the amine groups are positively charged and chitosan is soluble while at pH above 6.5, the amines are deprotonated and chitosan becomes insoluble (Yaneva et al., 2017). Meanwhile, for xanthan gum, the presence of *d*-glucosyl, *d*-mannosyl and *d*-glucuronyl acid residues and proportions of *O*-acetyl and pyruvyl residues causes changes in its charges. When pH > 4.5, the deprotonation of the acid residues occur, thereby increasing the negative charge density of the polysaccharide (Bueno & Petri, 2014).

Based on the obtained results, the addition of glycerol did not significantly affect the pH of xanthan gum, guar gum, and chitosan coatings, thus we can assure that the previously stated charges of the polysaccharides were maintained in the edible coatings when applied to the fresh-cut lotus root.

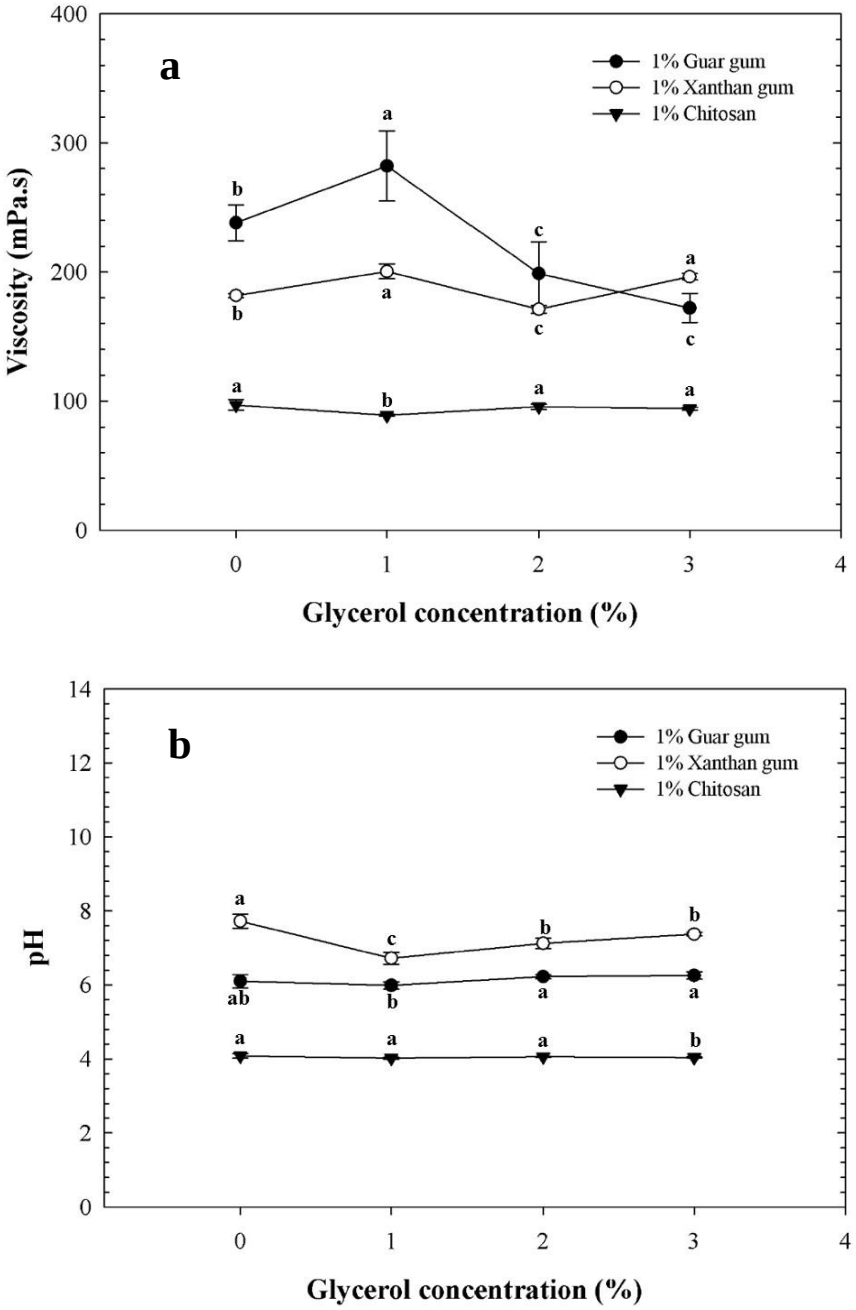


Fig. 2.2 Effect of different concentrations of glycerol (1, 2, and 3%) on the viscosity (a) and pH (b) of 1% polysaccharide-based edible coatings. Different letters indicate significant differences between different concentrations of glycerol (Duncan's test; $p < 0.05$).

2.3.2. Characteristics of the polysaccharide-based coatings for fresh-cut lotus root application

To assure the efficient functionality and barrier properties of these coatings, microscopic observations, and other physico-chemical characteristics of the coatings, such as Zeta potential, viscosity, pH, and contact angle, were evaluated before its application to fresh-cut lotus root (Table 2.1). Initially, the coating solutions were optically examined to evaluate agglomeration and homogeneity (Fig. 2.3). Due to the water solubility and compatibility of glycerol as a plasticizer to polysaccharide coatings, we observed that xanthan gum, guar gum, and chitosan coatings were homogenous, and the coatings were free from gas bubbles, which could interfere and affect the barrier properties of the coatings.

In this study, the author also confirmed that xanthan gum, guar gum and chitosan possessed different charges, as reported previously, which is needed for the application of LbL electrostatic deposition technique. Viscosity values were also measured for 1% guar gum, 1% xanthan gum and 1% chitosan edible coatings, as shown earlier. Our viscosity results were similar with Launay et al. (1997) where guar gum had higher viscosity (282 mPa.s) than xanthan gum (200.30 mPa.s). On the other hand, chitosan had a lower viscosity compared to both gums (88.87 mPa.s).

The author also examined the contact angle of the edible coatings. Contact angle measurements are important in surface and wetting properties of polymer coatings (Farris et al., 2011). Low contact angle values ($0° < \theta < 90°$) indicate high wettability of coatings, therefore may spread on the surface of the food material easily while large contact angles indicate low wettability thus the coatings tend to shrink on the food sample surface (Choi et al., 2002). In our study, the contact angles of the guar gum, xanthan gum, and chitosan

coatings ranged between 64° and 76°. The obtained relatively low contact angle values indicate that coatings based on these polysaccharides can easily cover the surface of the fresh-cut lotus root.

Finally, the formation of the edible coatings on the fresh-cut lotus root surface during day 0 was investigated through the scanning electron microscopy (Fig. 2.4). In the non-coated samples, the cell wall and several spherical shaped-parts of the fresh-cut lotus root (Fig. 2.4a), which we have speculated as the starch structures, were visibly seen. This clearly showed that without any coating barrier, the cell structures of the fresh-cut lotus root were exposed which can result to further oxidation of the samples during storage. However, the application of edible coatings has partially or completely covered the exposed cell structures which improved the barrier properties of the coatings and the storage stability of fresh-cut lotus root (Fig. 2.4b-f). For the single layers of 1% xanthan gum, 1% guar gum, and 1% chitosan (Fig. 2.4b-d), we observed partial covering only of the edible coatings on the surface, while for the LbL coatings of xanthan gum + chitosan (XG + Ch) and guar gum + chitosan (GG + Ch), we detected complete covering on the surface of the fresh-cut lotus root (Fig. 2.4e-f). Significant results were also observed for XG + Ch coatings, which showed complete covering on the surface of the fresh-cut lotus root and the possible formation of the XG + Ch complex, as indicated by the arrow (Fig. 4e). The coated samples of XG + Ch had also smoother surface as compared to the GG + Ch. Despite the presence of the GG + Ch coatings on the surface of the samples, distinct cracks were observed which could reduce the barrier properties of these coatings during the storage of fresh-cut lotus root.

Table 2.1. Physico-chemical properties of the coating treatments used in the study.

Coating treatment (w/w)	Zeta Potential [mV]	Viscosity [mPa.s]	pH [-]	Contact Angle [deg]
1% Guar Gum	-2.66 ± 0.11	282 ± 27.18	5.99 ± 0.09	64.97 ± 0.97
1% Xanthan Gum	-67.33 ± 3.32	200.3 ± 5.86	6.72 ± 0.16	76.45 ± 3.37
1% Chitosan	68.37 ± 2.15	88.87 ± 0.76	4.02 ± 0.03	66. 83 ± 2.60

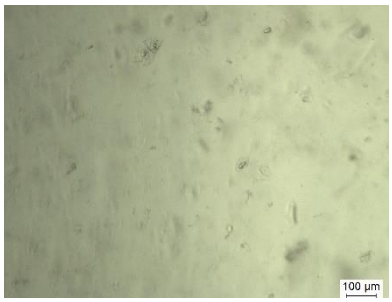
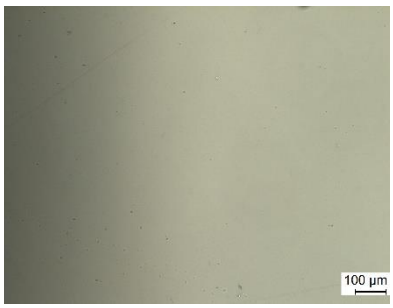
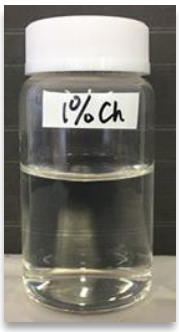
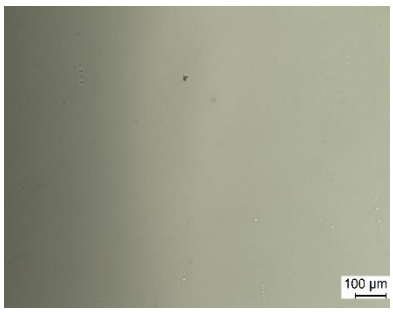
Coating Treatment		Micrographs
1% Guar Gum		
1% Xanthan Gum		
1% Chitosan		

Fig. 2.3 Optical micrographs of freshly prepared coatings treatments at room temperature.

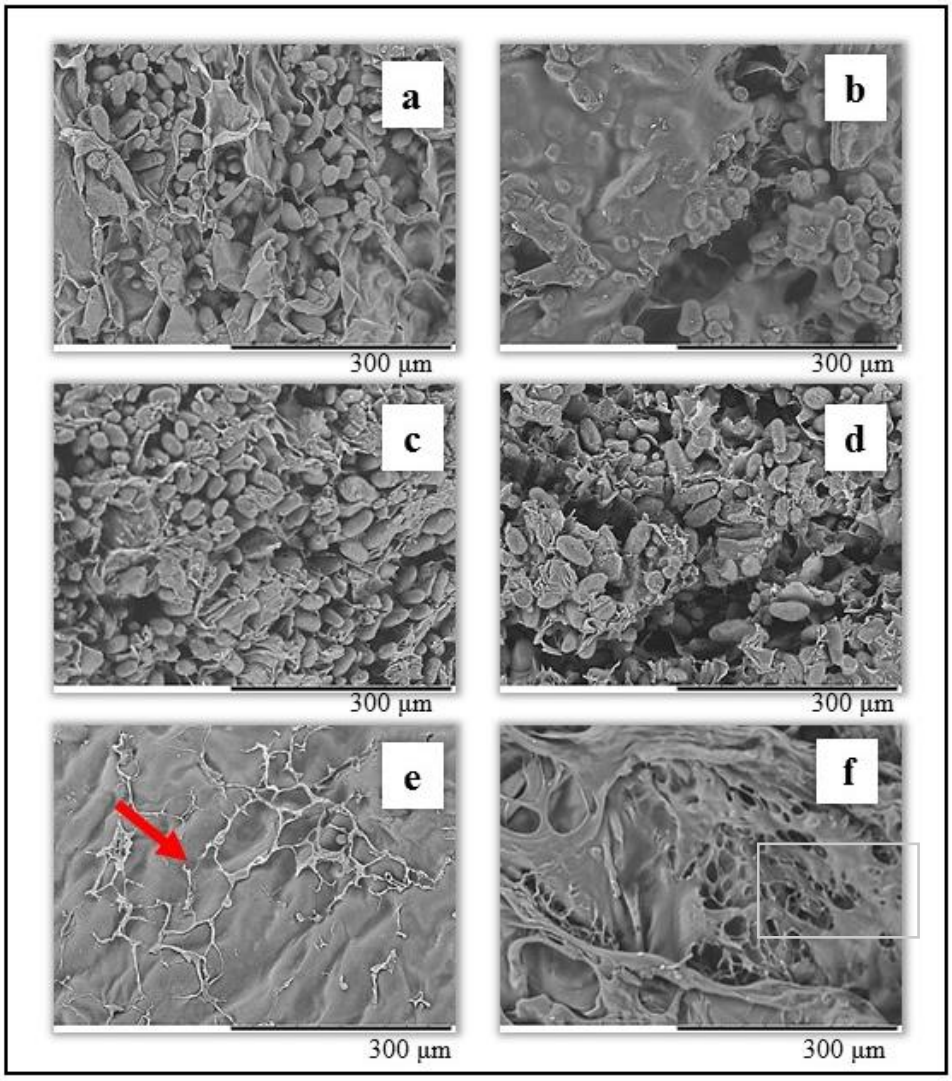


Fig. 2.4 SEM micrographs of edible coatings on the surface of fresh-cut lotus root at day 0 for: (a) control; (b) xanthan gum only; (c) guar gum only; (d) chitosan only; (e) xanthan gum + chitosan; (f) guar gum + chitosan

2.3.3. Effect of edible coatings on the quality of fresh-cut lotus root during storage

2.3.3.1. 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 2.5a 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 ($p > 0.05$) were 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

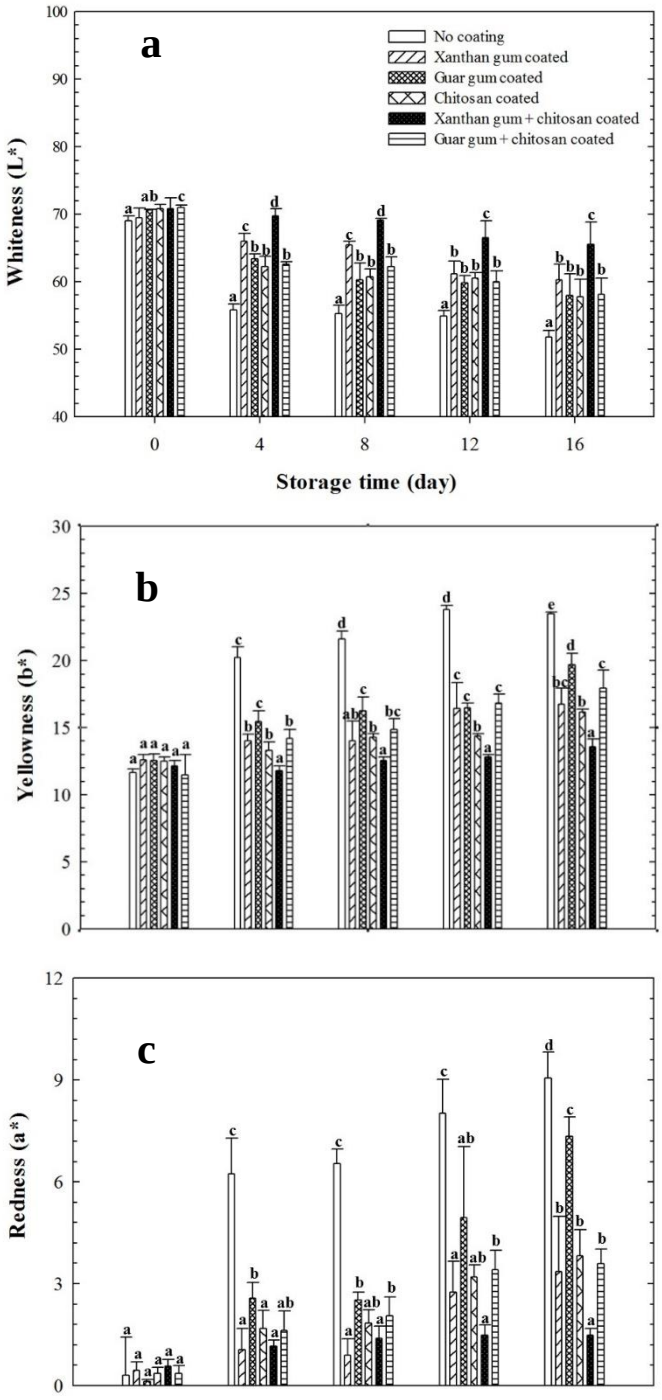


Fig. 2.5 Color changes of fresh-cut lotus root. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

(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, 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. 2.5b and Fig. 2.5c, 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.

2.3.3.2. Changes in texture of coated lotus root samples upon storage

Tissue softening in fresh-cut fruits and vegetables occurs due to the disruption of sub-cellular compartmentalization (Toivonen & Brummell, 2008). Figure 2.6 shows the effect of the different edible coating treatments on the texture, as indicated by hardness, of fresh-cut lotus root samples during 16 days of storage.

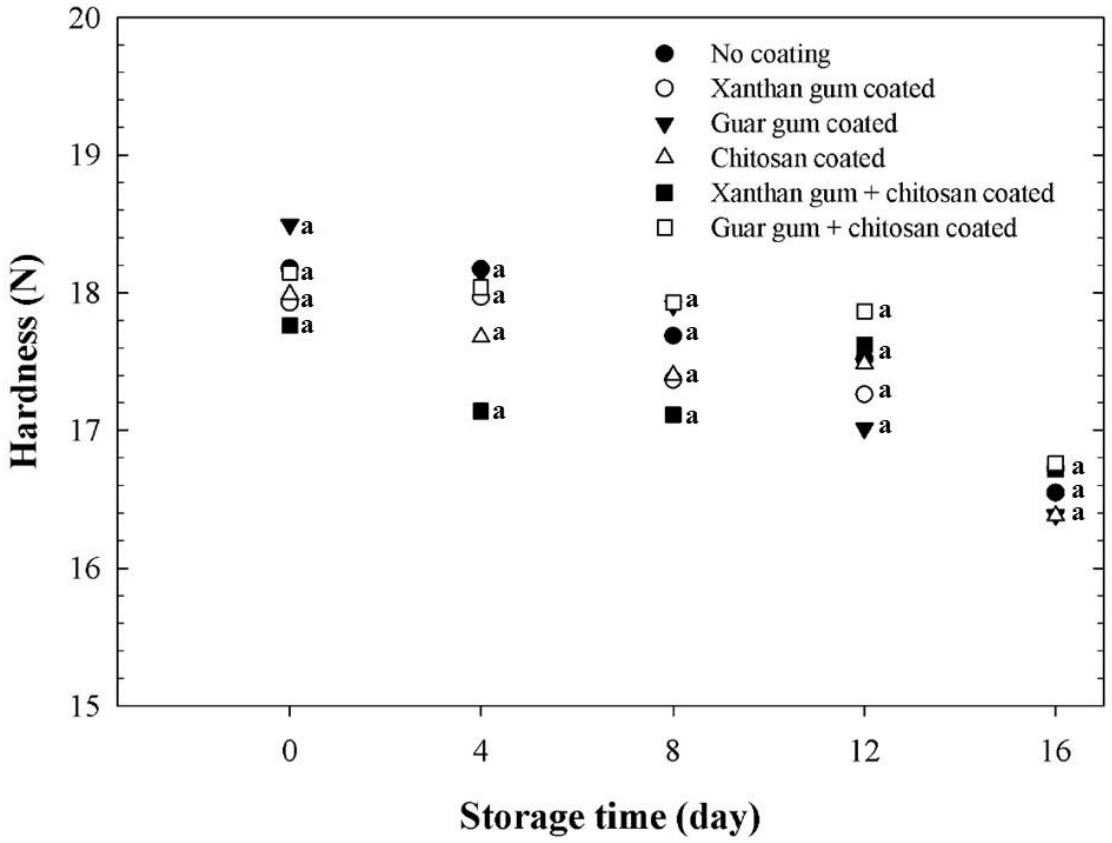


Fig. 2.6 Textural changes in fresh-cut lotus root during storage at 5 °C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

Decreasing hardness values in all samples (Fig. 6) indicated tissue softening. The decreasing hardness values during storage can be attributed to weight loss associated with water loss (Bico et al., 2009) . However, according to these results, the changes in hardness values during storage were not significant ($p > 0.05$) for the coated samples, which implies that the coatings act as a barrier against water and moisture migration. After 16 days of storage, coated fresh-cut lotus root samples had higher hardness values, which implies that the edible coatings can maintain texture of samples during storage. It has been recognized that edible coatings physically enhance the structure of fresh-cut products and decrease texture degradation rates (Poverenov et al., 2014). Previously, it was reported that tara gum based coatings applied on peaches allowed firmness maintenance during storage (Pizato et al., 2013)

In addition, morphological analyses (Fig. 2.7) can also validate the hardness results. The degradation of the microstructure of food products is very important in maintaining food quality (Tu et al., 2015). In this study, the author evaluated the effect of edible coatings on maintaining the integrity of the cell structure (Fig. 2.7). From the micrographs, it has been observed that all cell walls of samples from the treatments remained intact, and cell wall structures were visibly defined during 16 days of storage. This could indicate that the edible coatings protected the samples from microstructure degradation due to the polymeric barrier created on the surface of the fresh-cut lotus root samples.

Day 0 **Day 16**

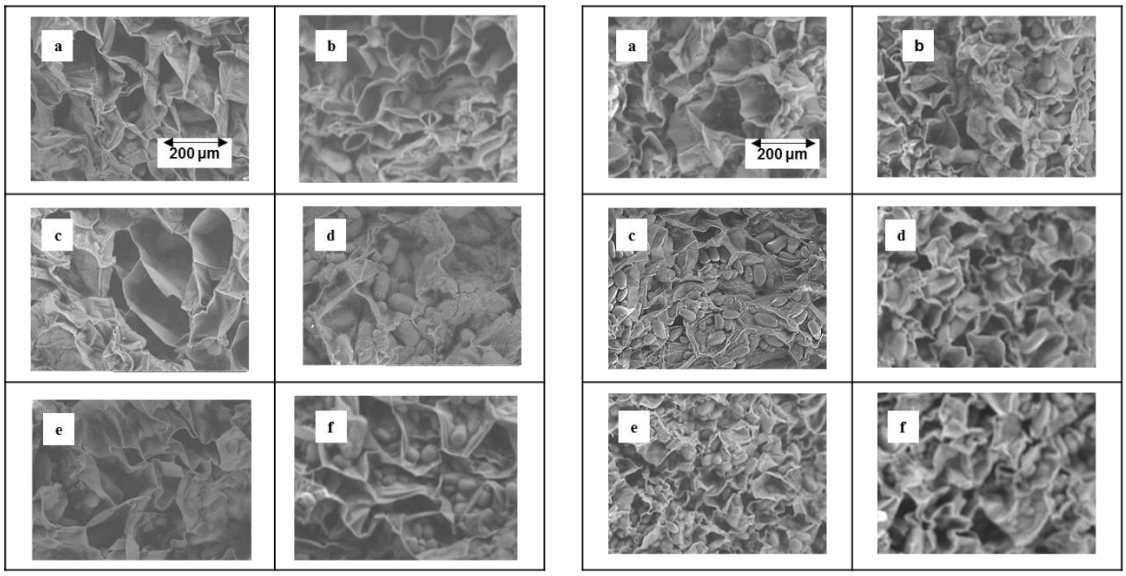


Fig. 2.7 SEM micrographs of fresh-cut lotus root at day 0 and day 16 upon storage at 5°C for: (a) control; (b) xanthan gum only; (c) guar gum only; (d) chitosan only; (e) xanthan gum + chitosan; (f) guar gum + chitosan

2.3.3.3. 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 were observed during 16 days of storage. However, it was found that after 4 days, weight loss of all coated samples was significantly lower compared to the untreated sample (Fig. 2.8). 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.

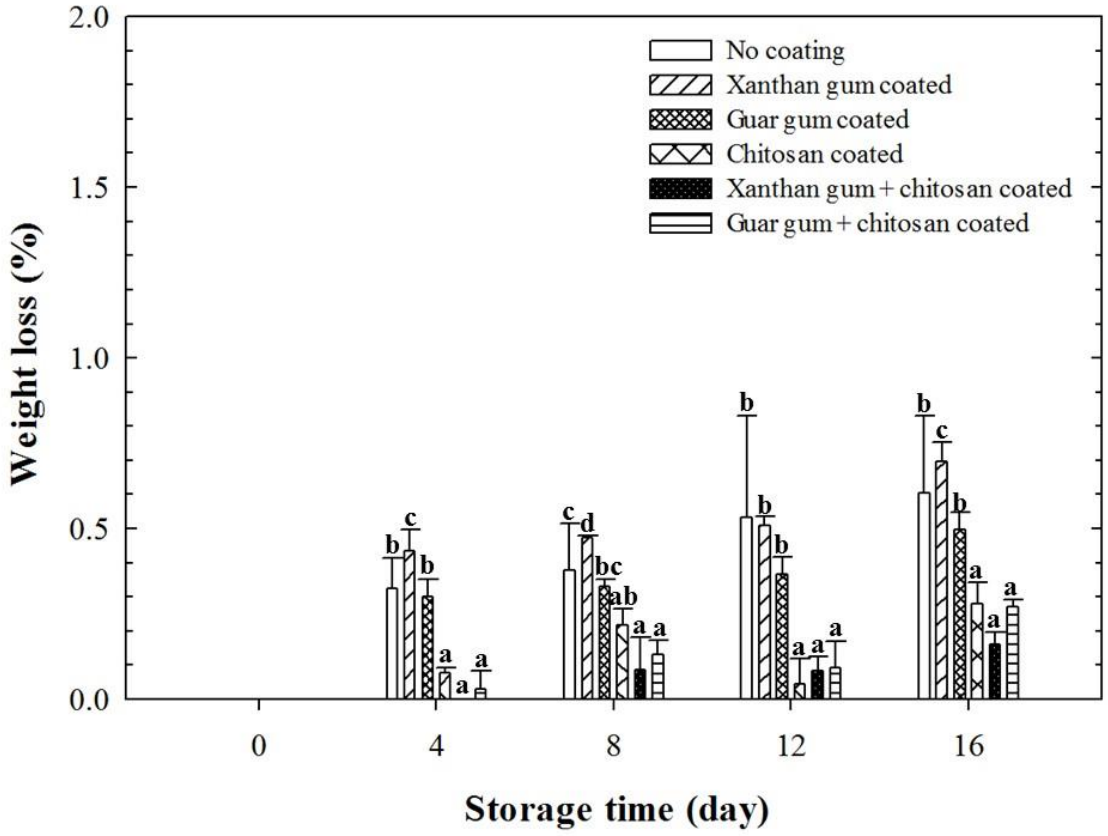


Fig. 2.8 Weight loss changes of fresh-cut lotus root during storage at 5 °C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

2.3.3.4. Changes in enzymatic activity of Polyphenol Oxidase (PPO) of coated lotus root samples upon storage

The degree of browning on the lotus root surface may be correlated to the PPO activity (Bico et al., 2009). The oxidation of the phenolic substrates by the PPO enzyme causes the browning of fresh-cut fruits and vegetables (Nguyen et al., 2003). Therefore, it is important to evaluate the enzymatic activity and correlate it with the browning of the fresh-cut lotus root. In this study, the author evaluated the effects of edible coatings on the enzymatic activity of the fresh-cut lotus root (Fig. 2.9). The auhtour found out that the PPO enzymatic activities of the fresh-cut lotus root treated with the edible coatings were significantly lower ($p < 0.05$) during storage as compared to the control throughout the storage period. These results for reduced PPO activity for the coated samples correlate with color changes with a Pearson coefficient, $r=-0.589$ at $p < 0.05$. This indicates that the maintained whiteness of the coated fresh-cut lotus root samples was due to the decreased activity of the PPO enzyme in the samples. The reduction of the PPO activity can be explained by the polymeric barrier created by the coatings, which reduces the enzymatic interaction with the atmospheric oxygen. Petriccione et al. (2015) showed similar results of the reduced effect of chitosan edible coatings on the PPO activities of the strawberry cultivars during storage and stated that the reduced enzymatic levels are due to the low oxygen availability contributed by the edible coatings. Meanwhile, the application of carbon monoxide of fresh-cut lotus root was also reported to decrease PPO enzyme activity during 8 days of storage (Zhang et al., 2013).

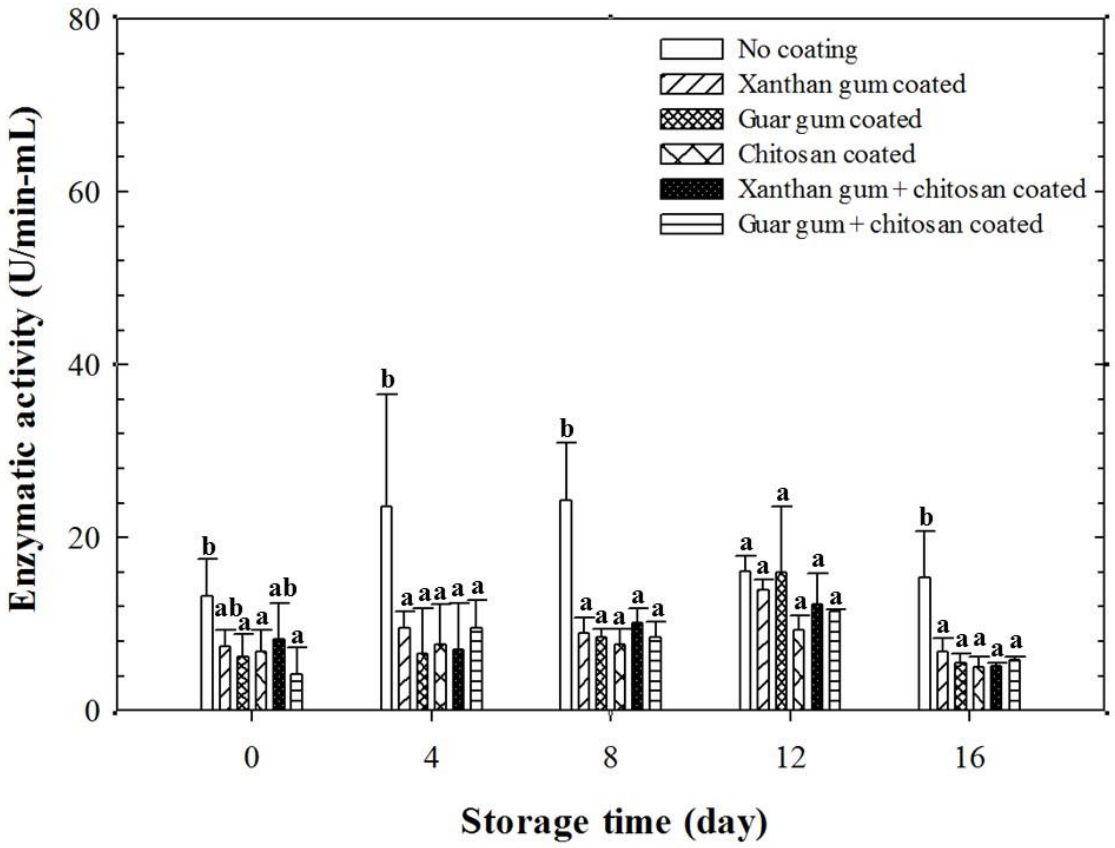


Fig. 2.9 PPO enzymatic activity of fresh-cut lotus root during storage at 5 °C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

2.3.3.5. Changes in total phenolic content of coated lotus root samples upon storage

Slicing during the fresh-cut lotus preparation is one of the abiotic stresses that affect the quality of the fresh-cut products (Hu et al., 2014), which further causes the increase of phenolic compounds (Reyes et al., 2003). Based on our results, we observed that all the fresh-cut lotus root samples increased in total phenolic content throughout the 16-day storage, as reported in Fig. 10. Wounding of tissues during fresh-cut processing can explain the increase of total phenolic contents in our samples (Hu et al., 2014). In this study, the author observed that there was no remarkable relationship between the total phenolic content and whiteness, with a correlation r value of 0.030 ($p < 0.05$). However, it was found that there was a low correlation between the total phenolic content and PPO activities, with an r value of 0.524 ($p < 0.05$), as affected by the application of edible coatings. Rocha & Morais (2002) also found similar results that there is a low correlation of the total phenolic content and enzymatic browning in minimally processed apples. However, the Folin-Ciocalteu method has been reported to have a low specificity for specific phenolic compounds, thus, could explain further the differences in the obtained total phenolic content results.

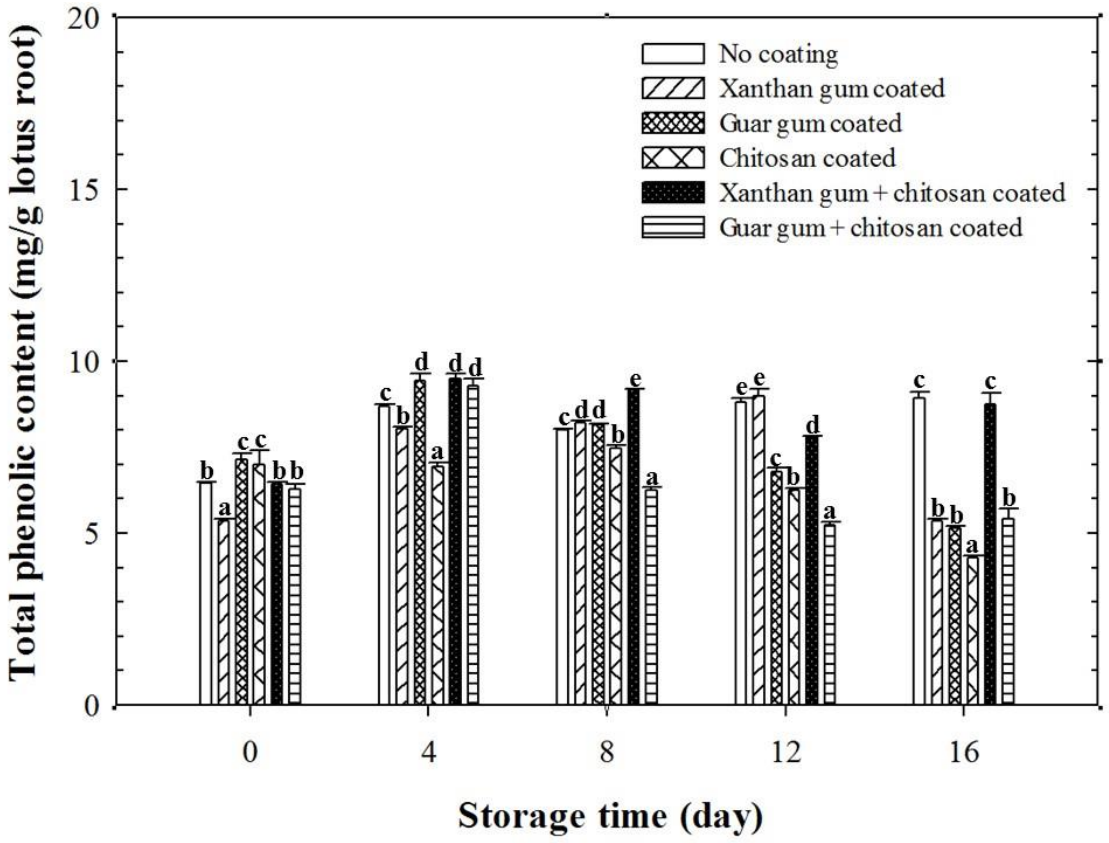


Fig. 2.10 Total phenolic content changes during storage at 5°C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

2.4. Conclusions

In this study, 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 was highlighted. Based on these 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, water vapor loss and PPO enzymatic activity. With the improved barrier properties of the LbL edible coatings, the storage stability of coated fresh-cut lotus root as compared to non-coated lotus root samples, in terms of color, texture, weight loss, PPO enzymatic activity, and morphological qualities were significantly improved during storage up to 16 days at 5°C. In addition, the improved performance of the LbL edible coatings were 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.

Chapter 3

Influence of Xanthan Gum Concentration on the Barrier Properties of Xanthan-Chitosan Edible Coating

3.1. Introduction

The recent increase in consumer demand for freshly prepared, healthy and convenient fruits and vegetables has resulted to the fast growth of the fresh-cut industry worldwide (Qadri et al., 2015). However, processing such as peeling, cutting and slicing lead to physical damage of these products, thus shortening their shelf-life and increasing their susceptibility to enzymatic browning, texture decay, microbial contamination and unpleasant volatile compound production (Khan et al, 2014; Tapia et al., 2008; Watada et al.,1996).

Lotus root (*Nelumbo nucifera*) is consumed worldwide and is known for its crispy texture, white color and attractive aroma and high nutritional content (Gao et al., 2017). However, fresh-cut lotus root is prone to enzymatic browning and as a result, deteriorates its quality and shortens its shelf-life. Sun et al. (2015) reported that enzymatic browning in fresh-cut products such as the lotus root, is a result of the polyphenol oxidase (PPO) and peroxidase (POD) action on polyphenols, which produce quinones. Various attempts from researchers to improve the shelf-life of fresh-cut lotus root have been outlined and have focused on chemicals methods such as the use antibrowning agents and preservatives and physical methods such as the application of gases and modified atmosphere packaging. Xing et al. (2012) evaluated on the combined effects of antibrowning agents, cinnamon oil fumigation and moderate vacuum packaging; Zhang et al. (2013) confirmed the efficacy of carbon monoxide on browning of fresh-cut lotus root; Son et al. (2015) accounted the synergistic use of antibrowning, heat treatment and modified atmosphere packaging (MAP); Sun et al. (2015) reported the application of hydrogen sulfide in retarding enzymatic browning of fresh-cut lotus root slices while Gao et al. (2017) determined the effects of 24-epibrassinolide on enzymatic browning and

antioxidant activity of fresh-cut lotus root slices. However, there were numerous reported safety concerns in the application of some chemical preservatives and the high-costs of modified atmosphere packaging (MAP) equipment, thus, one of the strategies we found is the application of edible coatings from gum polysaccharides, which we believe to be a promising technique in enhancing the shelf-life of fresh-cut lotus root. Edible coatings are regarded as feasible and alternative technology which can impart protection, improve appearance, lessen respiration rates, enzymatic activities and water losses (Silva-Vera et al., 2018). The efficacy of edible coatings can be explained by the creation of a modified atmosphere, almost similar to that of MAP, which results to a semi-permeable barrier against gases and moisture (Ali & Maqbool, 2010).

In general, edible coatings are commonly applied as single layers on the surface of fresh-cut products. However, Poverenov et al. (2014) found that single layers of these coating materials were not sufficient to protect food products against oxidation and moisture migration, thus some studies have been investigating the improved barrier effects of bilayer or multicomponent coatings. The layer-by-layer (LbL) electrostatic deposition of charges is one of interesting approaches for the application of coatings. For instance, Yan et al. (2019) reported that the layer-by-layer coatings from chitosan and carboxymethylcellulose (CMC) maintained the firmness and volatile compounds of strawberries during storage while Souza et al. (2014) accounted the use of layer-by-layer alginate and chitosan edible coatings in enhancing the microbiological and physico-chemical properties of fresh-cut mangoes during storage.

Previously, the author found that the layer-by-layer electrostatic deposition of the positively charged chitosan and negatively charged xanthan gum coatings decreased browning, polyphenol oxidase (PPO) enzyme activity, weight loss and morphological

changes on fresh-cut lotus root during storage for 16 days at 5°C. It has been reported that the hydrogel network from the ionic interactions between the amino groups of chitosan and the carboxyl groups of xanthan gum showed unique swelling characteristics that could efficiently control the release of encapsulated materials such as therapeutic agents, enzymes and bacteria (Argin-Soysal et al., 2009). For example, Merlusca et al. (2016) reported the use of the xanthan gum-chitosan complex for the encapsulation of *Lactobacillus acidophilus* for the preparation of insulin-based nanoparticles while Chu et al. (1996) applied the xanthan gum-chitosan polyelectrolyte complex in the immobilization of *Corynebacterium glutamicum*. Moreover, the formation of a strong xanthan gum and chitosan network is highly dependent on the different complexation conditions such as chitosan solution pH, polymer concentration, complexation time, and mixing ratio (Argin-Soysal et al., 2009).

As far as the author knows, there were no reported studies yet on the effect of layer-by-layer electrostatically deposited edible coatings of xanthan gum and chitosan for improving the storage stability of fresh-cut lotus root during storage, and moreover, the effect of complexation parameters such as the polymer concentration, on the barrier properties of these edible coatings on fresh-cut products. Therefore, the author evaluated the effect of increasing the xanthan gum concentration in improving the barrier properties of the xanthan gum-chitosan layer-by-layer electrostatically deposited edible coatings on the fresh-cut lotus root for 16 days storage at 5°C.

3.2. Materials and methods

3.2.1. Chemicals

Chemical reagents such as xanthan gum, chitosan, polyvinylpyrrolidone, catechol, sodium phosphate, Folin-Ciocalteu and gallic acid were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan).

3.2.2. Plant material

The lotus root (*N. nucifera*) samples used in our study were bought from a local supermarket (Tsukuba, Japan). The whiteness and length of duration in the supermarket shelf were evaluated prior to purchase to ensure that the samples were fresh and of good quality. The samples were washed completely in running tap water, peeled and sliced manually into 5 mm thickness.

3.2.3. Preparation of the polysaccharide coating solutions

Xanthan gum and chitosan aqueous solutions were prepared by dissolving xanthan gum and chitosan powder separately in Milli-Q water. In this study, 0.5 g, 1 g, and 1.5 g of xanthan gum powder were dissolved in 99.5 mL, 99 mL, and 98.5 mL of Milli-Q water to achieve 0.5%, 1% and 1.5% xanthan gum aqueous solutions while 1 g of chitosan powder was dissolved in 99 mL of 1% (w/w) acetic acid solution (pH=2.22) to achieve 1% concentration of chitosan coating solution. Xanthan gum and chitosan solutions were magnetically stirred for 2 h at 25°C (HS 360 H, As One Corporation, Tokyo, Japan), for the complete dissolution of the powder. Glycerol at 1% (w/w) was added as plasticizer for the edible coatings. Previously, we found that glycerol was the most compatible

plasticizer for the xanthan gum and chitosan coatings. Preliminary experiments were performed to determine the appropriate plasticizer for the edible coatings, and it has been found out that glycerol was the most compatible plasticizer for the xanthan gum and chitosan coatings. The viscosity of the coating solutions were measured prior to the application on the fresh-cut lotus root samples.

3.2.4. Plant material and processing conditions

Four experimental setups consisting of no coating (control), 0.5% xanthan gum + chitosan, 1.0% xanthan gum + chitosan, and 1.5% xanthan gum + chitosan were prepared in our study. The control treatments were composed of 5-mm thick, freshly-cut lotus root without the application of any treatment. The three other treatments were the layer-by-layer electrostatically deposited coatings of xanthan gum (0.5% w/w, 1.0% w/w and 1.5% w/w) + 1% w/w chitosan. Prior to the experiments, the zeta-potential of xanthan gum and chitosan were measured to confirm the opposing charges of xanthan gum and chitosan using the dynamic light scattering technique.

The edible coatings of xanthan gum and chitosan were applied thru the layer-by-layer electrostatic deposition of charges dipping method (Fig. 3.1). The fresh-cut lotus root slices were dipped in the first layer of coating solution of xanthan gum with varying concentrations (0.5%, 1.0% and 1.5% w/w) for 2 mins, dried naturally in room temperature for 1 min and then dipped in the second layer of chitosan solution for 2 mins. The samples were dried naturally for 1 min and then packed per treatment in polyethylene bags (60 μm x160 mm x 260 mm; GT-1626, Kurilon, Sigma Tube, Tokyo, Japan) and were stored for 16 days at 5 °C for color, enzymatic activity, and morphological tests.

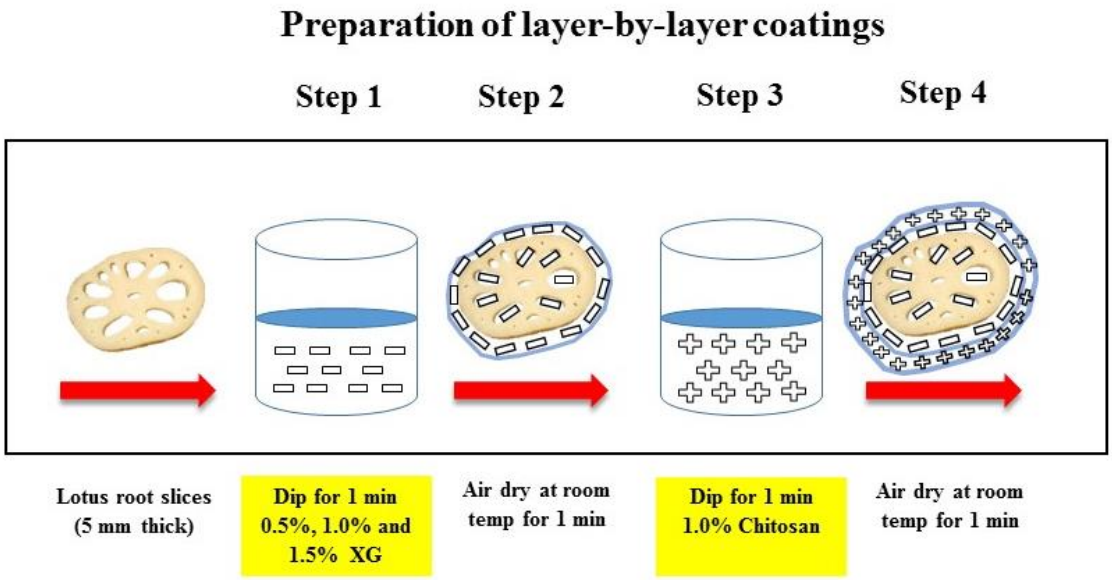


Fig. 3.1 Schematic presentation of the electrostatic deposition applied as layer-by-layer edible coatings to fresh-cut lotus root. (XG: Xanthan gum)

3.2.5. Analysis of the polysaccharide coating solutions

3.2.5.1. Viscosity

The viscosity of the coating solutions from 0.5%, 1%, and 1.5% xanthan gum were analyzed using a Vibro Viscometer (SV-10, A & D Company Ltd., Tokyo, Japan) at 25°C. Thirty-five (35) mL of the liquid sample was placed in the vessel for measurement.

3.2.5.2. Zeta Potential

The confirmation of the charges of the polysaccharides for the layer-by-layer electrostatic deposition was confirmed through the measurement of the Zeta-potential (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK) using the dynamic light scattering technique. The samples were loaded in a folded capillary cell and were automatically measured using the instrument. The charges of the polysaccharides, as measured by the zeta-potential values, were -67.33 mV for 1% xanthan gum aqueous solution while +68.37 for 1% chitosan aqueous solution.

3.2.5.3. Amount of xanthan coating

The effect of the xanthan gum concentration on the amount of coating material adhered on the fresh-cut lotus root samples were evaluated using the method of measurement of Zevallos and Krochta (2003). The fresh-cut lotus root samples were held using clips and were dipped in the 0.5%, 1% and 1.5% xanthan gum solutions for 2 mins and then rapidly removed and drained over the glass beaker containing the xanthan gum solution. The glass beaker was placed on top of an analytical balance (Shimadzu Corporation UX 620H, Unibloc Toploading Balance, Kyoto, Japan) to measure the

xanthan gum solution weight versus draining time. The amounts of the xanthan gum solution on the fresh-cut lotus root at 0, 20 s, 40 s, 60 s, 80 s, 100 s, 120 s, 140 s and 160 s draining time was the difference between the initial weight of the xanthan gum solution in the glass beaker and the weight recorded at the respective draining times.

3.2.5.4. Coating formation

The coating formation of the polysaccharide-based coating solutions were investigated through the scanning electron microscope (SEM miniscope 1000, Hitachi, Tokyo, Japan). Prior to microscopy analysis, the coated and non-coated samples were frozen for 24 h and freeze-dried for 24-48 h (FDU-2100 Eyela Desktop Freeze Dryer, Tokyo, Japan). After freeze drying, the samples were mounted on an aluminum sample probe for magnification at 300×.

3.2.6. Evaluation of physico-chemical changes during storage

3.2.6.1. Color

The color changes during 16 days of storage at 5°C were evaluated using spectrophotometer (CM-5, Konica Minolta, Tokyo, Japan). It was decided to keep the storage time up to 16 days to provide enough time to assess the color and enzymatic changes and be able to identify the stage at which enzymatic browning occurs in the fresh-cut lotus root after the application of the coating treatments. The CIE L*a*b* values were used to express different color changes in the coated and non-coated fresh-cut lotus root samples. The L* values indicate lightness or darkness, a* for redness and b* for

yellowness. Using the L*, a*, and b* values, the total color changes (ΔE) were calculated based on the following equation 3.1, as reported by Supapvanich et al. (2016):

$$(\Delta E) = [(L_2^*-L_1^*)^2 + (a_2^*-a_1^*)^2 + (b_2^*-b_1^*)^2]^{1/2} \quad (3.1)$$

Browning indices were also computed using equations 3.2 and 3.3, based on Kortei et al. (2015):

$$\text{Browning index (BI)} = \frac{[100-(x-0.31)]}{0.17} \quad (3.2)$$

where:

$$x = \frac{(a^* + 1.75\,L^*)}{5.645\,L^* + a^* - 3.012\,b^*} \quad (3.3)$$

3.2.6.2. Polyphenol oxidase activity (PPO)

The enzymatic activity of polyphenol oxidase (PPO) enzyme was determined based on the method of Son et al. (2015) with few modifications. Ten grams of each fresh-cut lotus root per treatment were homogenized manually using mortar and pestle with sodium phosphate buffer (0.2 mol/L, pH 7.0 with 2% polyvinylpyrrolidone) following a ratio of 2.0 mL of buffer per 1 g of lotus root in an ice bath. The homogenates were centrifuged (high-speed refrigerated centrifuge, Tomy MX -307, Tomy Seiko, Tokyo, Japan) at 12000 × *g* for 10 min at 4 °C. The supernatant, which was used further for analysis, was separated from the filtrate by decanting the liquid to another centrifuge tube. The enzymatic activity was determined by the increase of absorbance at 410 nm for catechol at 25 °C using a spectrophotometer (UV-Vis, V-530, JASCO Inc., Tokyo, Japan). The 0.2 mL supernatant was added with 2.8 mL of the catechol substrate solution (0.02 mol/L catechol in 0.05 mol/L sodium phosphate buffer, pH 7). The catechol substrate solution (catechol in sodium phosphate buffer) was used as the reference. The enzymatic

activity (units/(min-mL enzyme)) was detected by the linear section of the activity curve. The 1 unit of PPO enzyme activity was defined as the change in 0.001 in the absorbance value per min.

3.3. Results and discussion

3.3.1. Characteristics of the polysaccharide-based coatings for fresh-cut lotus root application

To assure the efficient functionality and barrier properties of the coatings, physico-chemical characteristics such as viscosity and thickness were evaluated before the application to fresh-cut lotus root samples (Table 3.1).

Table 3.1. Influence of xanthan gum concentration on the thickness of coatings on fresh-cut lotus root.

Xanthan gum concentration	Viscosity (mPa.s)	Coating thickness (mm)
0.5	62.3 ± 0.4^{a}	0.37 ± 0.001^{a}
1	243.33 ± 2.89^{b}	1.27 ± 0.02^{b}
1.5	442.33 ± 20.53^{c}	3.36 ± 0.04^{c}

The results showed that an increase in xanthan gum concentration from 0.50% (w/w) to 1.50% (w/w) resulted to an increase in the xanthan gum solution viscosity from 62.3 mPa.s to 442.33 mPa.s. The author observed that an increase in the viscosity of the coating solutions significantly increased the thickness of the coatings in the fresh-cut lotus root (Fig. 3.2). Previous studies of Zevallos & Krochta (2003) stated that the the average thickness and the amount of liquid coating adhered to coated apple samples were dependent on viscosity, draining time and density of the biopolymer solutions. In this work, the author also investigated the effect of draining time on the amount of coatings adhered on the surface of the fresh-cut lotus root samples (Fig. 3.3) and found a decrease in coating material from 0 secs to 40 secs of draining time. After 60 seconds draining time, no changes have been observed there were no observed changes in the amount of coating adhered on the lotus root surface in 0.5%, 1.0% and 1.5% xanthan gum treatments, therefore, it was decided to drain the first coating layer of xanthan gum for 60 secs (1 min) before applying the second layer of chitosan coating. Among all the treatments, 1.5% xanthan gum coatings resulted to the highest amount of adhered coating material in the fresh-cut lotus root which signify that increasing the xanthan concentration could increase the coating material and resulting thickness on the samples, thus, may prevent the oxidation of the fresh-cut lotus root samples.

In addition, the author also investigated the effect of xanthan gum concentration on the formation of the xanthan gum and chitosan complex (Fig. 3.4). It was found that 1.5% xanthan gum resulted to the formation of a complete xanthan gum and chitosan network as compared to the 0.5% and 1.0% xanthan gum. Argin-Soysal et al. (2009) reported that xanthan gum concentrations more than 1.5% (w/v) formed amorphous capsules while lesser concentrations of xanthan gum below 0.5% (w/v) resulted to

unstructured capsules. Two glass transition temperatures were also observed in solutions with 0.7% (w/v) xanthan gum concentrations.

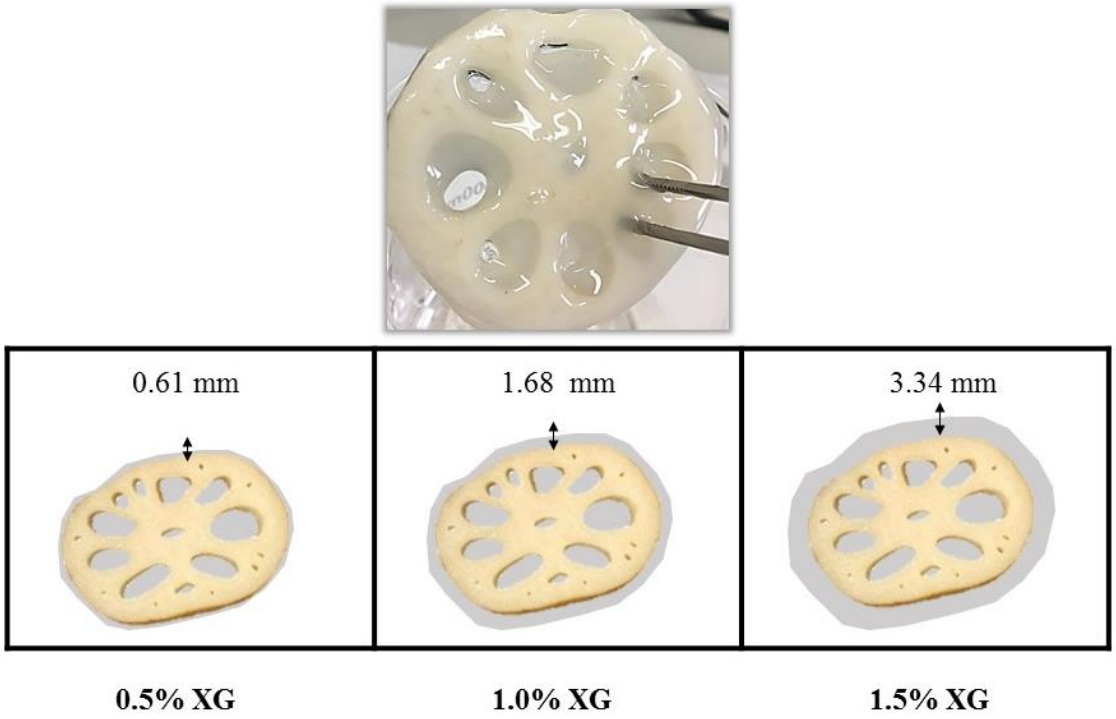


Fig. 3.2 Effect of increasing concentration of xanthan gum on the resulting coating thickness on fresh-cut lotus root.

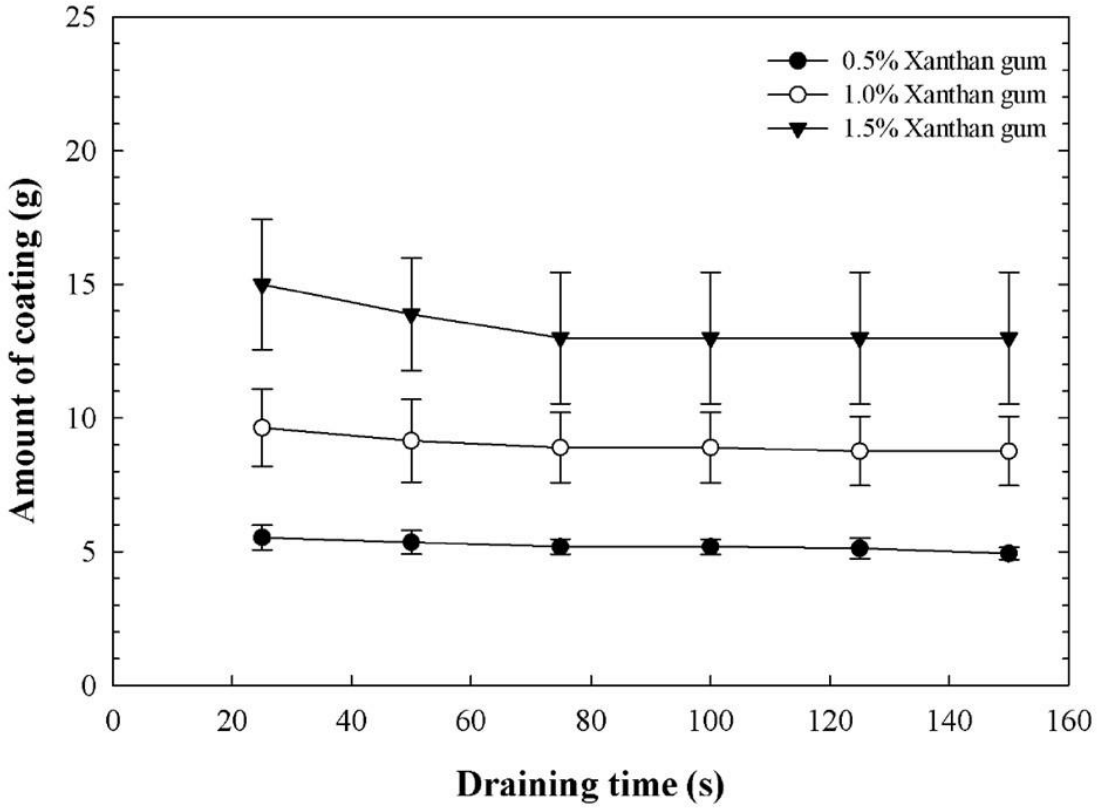


Fig. 3.3 Draining time effects on the amount of xanthan gum coatings applied to fresh-cut lotus root.

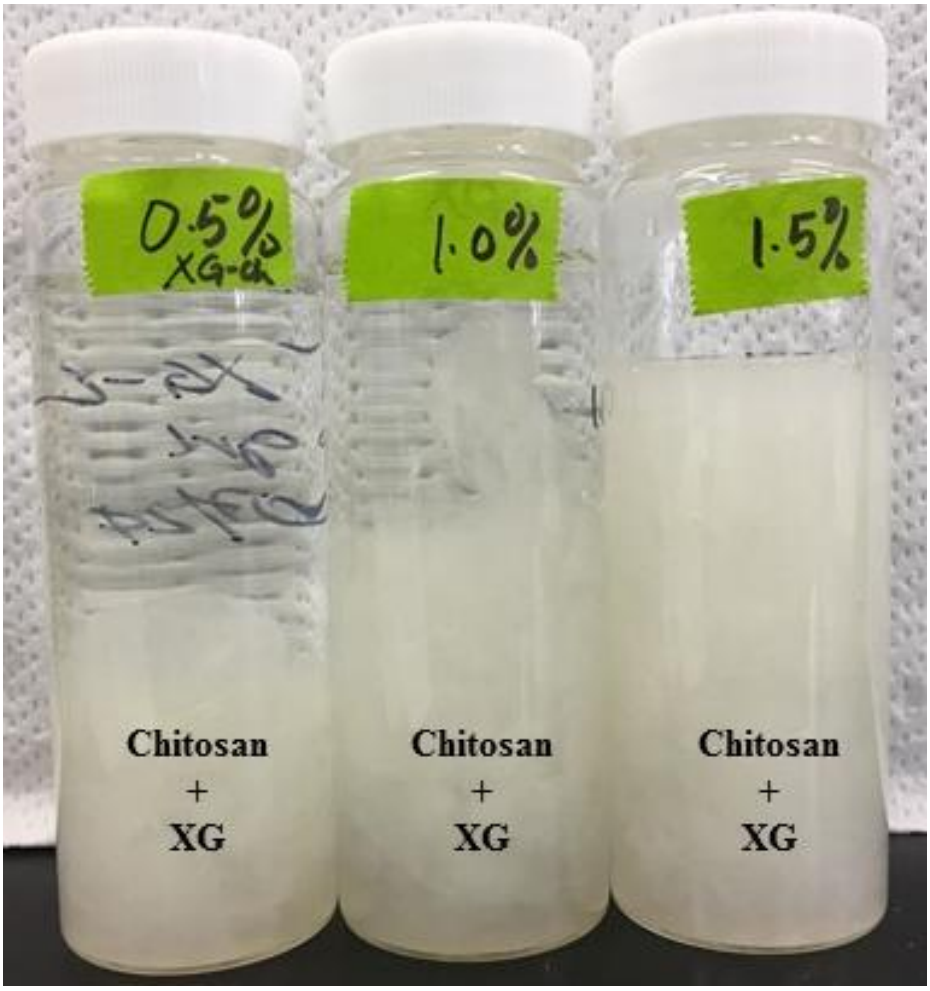


Fig. 3.4 Effect of amount of xanthan gum on the xanthan gum and chitosan interaction.

indicating for the presence of uncrosslinked xanthan gum and chitosan in the polymer network. However, with an increase in xanthan gum concentration up to 1.0% (w/v), the two glass transition temperatures were perceived to disappear, suggesting a complete crosslinking of the two polymers.

Finally, the formation of edible coatings on the fresh-cut lotus root surface during day 0 were also highlighted in this work using the scanning electron microscopy (Fig. 3.5). Initially, it was observed that the cell wall and several spherical shaped-parts of the fresh-cut lotus root, which we have speculated as the starch structures, were visibly seen in the non-coated samples. Without a coating barrier, the cell structures of the fresh-cut lotus root were exposed, thus, may possibly lead to oxidation of the samples during storage. In contrast with the application of the xanthan gum and chitosan edible coatings, a complete covering of the fresh-cut lotus root surface was observed, which may possibly enhance the barrier properties of the coatings and the storage stability of the fresh-cut lotus toot during storage for 16 days at 5°C. Based on these results, it was found that increasing the xanthan gum concentration from 0.5% (w/v) to 1.5% (w/v) formed smoother surface of xanthan gum and chitosan edible coatings on the surface of fresh-cut lotus root. For 0.5% xanthan gum coatings, we observed for protruded areas on the surface of the fresh-cut lotus root which suggested the partial covering of the starch structures whereas these protruded areas disappeared when we increased the xanthan gum concentration to 1.0% (w/w) and 1.5% (w/w). The author speculated that due to the higher concentration of xanthan gum in the xanthan gum-chitosan complex, a stronger crosslinking network between xanthan gum and chitosan occurred, therefore, a

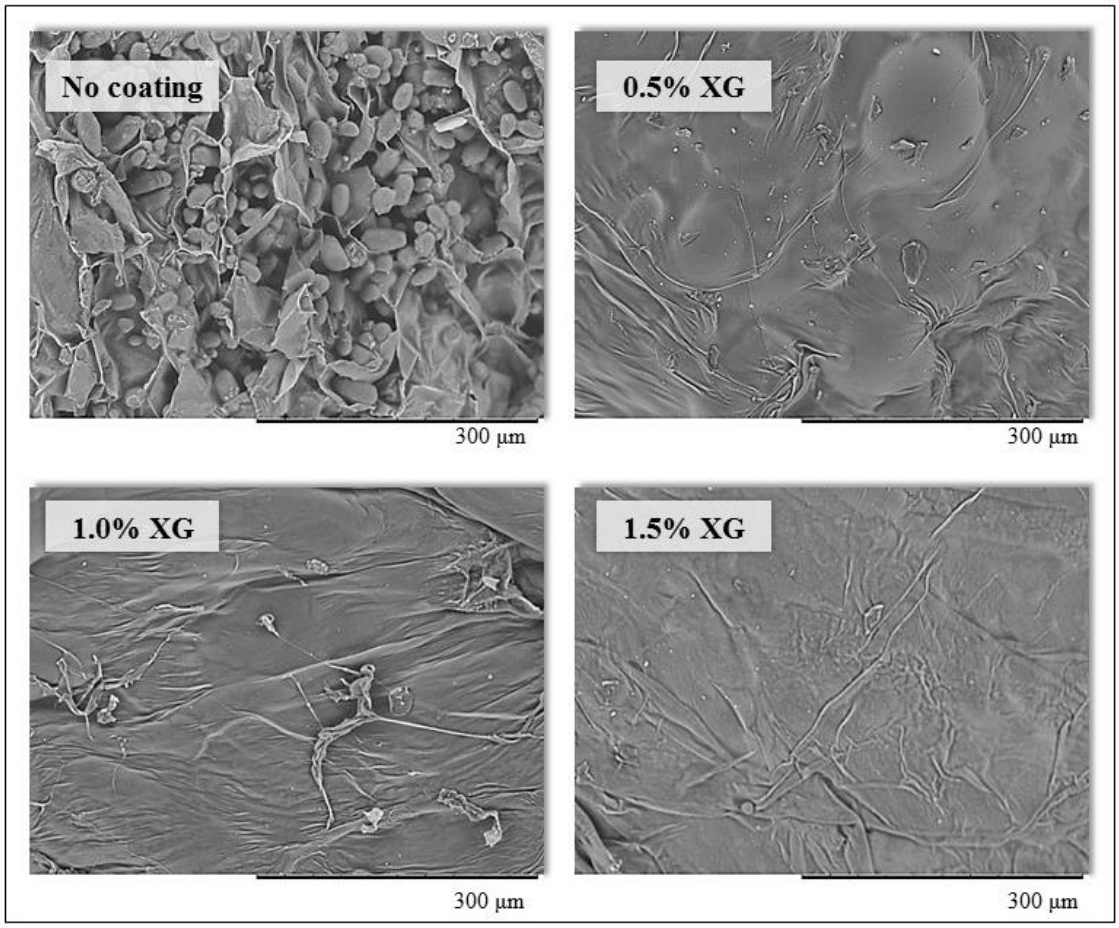


Fig. 3.5 SEM micrographs of xanthan gum-chitosan coatings on the surface of fresh-cut lotus root at day 0.

stronger coating layer was formed and prominently covered the complete fresh-cut lotus root surface. No distinct cracks were observed for the 1.0% (w/w) and 1.5% (w/w) which could prevent the entry of gases such as oxygen and carbon dioxide and may potentially improve the coating's barrier functionality to oxidation and to water and solute migration.

3.3.2. Effect of edible coatings on the storage stability of fresh-cut lotus root during storage

3.3.2.1. Changes in color of coated - lotus root samples upon storage

Enzymatic browning is one of the limiting factors on the shelf-life of fresh-cut products which leads to discoloration, nutrient loss and undesirable flavor production. The color qualities of a food product is an important consideration for selection and purchase (Sharma, et al., 2019; Du, et al., 2009). The whiteness, as described by the L* values, were reported in Fig. 3.6a. Higher whiteness values (L*), suggested a brighter surface of the sample. During the storage time, the whiteness values generally decreased because of the occurrence of enzymatic browning especially for the non-coated fresh-cut lotus root samples. After 4 days of storage, we observed that the whiteness values of the non-coated samples decreased significantly ($p < 0.05$) as compared to the coated samples, implying the occurrence of enzymatic browning during storage. The L* values of the samples coated with 0.5%, 1.0% and 1.5% xanthan gum were significantly higher ($p < 0.05$) than that of the non-coated samples during the 16 days of storage. Among all the coated samples, we found that the samples coated with 1.5% xanthan gum resulted to significantly higher L* values ($p < 0.05$) as compared to other coated treatments. Sharma & Rao (2015) presented the similar results that 2.5% xanthan gum coatings enriched with

1% cinnamic acid decreased the changes of L* values during the 8 days storage of fresh-cut pears indicating the efficacy of xanthan gum coatings to prevent oxidation. Meanwhile, the improved color qualities of the fresh-cut lotus root samples coated with 1.5% xanthan gum during storage can be attributed to the stronger complex formed between xanthan gum and chitosan with the following xanthan gum concentration level. Argin-Soysal et al. (2009) reported that with an increasing xanthan gum concentration up to 1.5% (w/v), glass transitions tend to disappear demonstrating a complete crosslinked network. However, when the xanthan gum concentration was decreased to 0.7% (w/v), the appearance of glass transitions were observed, suggesting an incomplete crosslinking of the xanthan gum and chitosan complex. The complete crosslinking of xanthan gum and chitosan at 1.5% (w/v) xanthan gum improved the barrier properties of the 1.5% xanthan gum edible coatings on its application to fresh-cut lotus root during storage. Likewise, higher thickness values (3.36 mm) were achieved when using 1.5% (w/v) xanthan gum, as compared to 0.5% and 1.0% (w/v) xanthan gum, whereas thickness of coatings were 0.37 mm and 1.27 mm, respectively (Table 1). An increase in the thickness of the xanthan gum and chitosan edible coatings, with the influence of an increase in xanthan gum concentration, may have improved barrier properties of the xanthan gum and chitosan edible coatings to fresh-cut lotus root.

Meanwhile, the redness (a*) and yellowness (b*) values of the coated and non-coated samples during storage were also investigated (Fig. 3.6b and 3.6c). Remarkable increasing trends of a* and b* values for the non-coated samples were observed which manifested color changes during storage. This was in contrast with 0.5%, 1.0% and 1.5% xanthan gum coated samples, having recorded minimal changes in color, which could be

mainly attributed to the improved barrier properties of the xanthan gum-chitosan edible coatings.

Using the reported L*, a* and b* values, the total color changes (ΔE) and browning index were calculated, as reported in Fig.7 and Fig.8. These results showed color changes were significantly reduced ($p < 0.05$) with the application of 0.5%, 1.0% and 1.5% xanthan gum coating treatments (Fig. 3.7). A significant reduction of 87.5% in total color changes were observed for samples coated with 1.5% (w/v) xanthan gum while 83.33% and 62.50% for 1.0% (w/v) and 0.5% (w/v) xanthan gum respectively. This evidently signified that the xanthan gum and chitosan edible coatings were effective in preventing oxidation and an increase in the concentration of xanthan gum in the xanthan gum and chitosan complex proved promising barrier benefits for improving the storage stability of fresh-cut lotus root during 16 days of storage. The calculated browning indices (Fig. 3.8) were also in agreement with these results, demonstrating that significant browning ($p < 0.05$) occurred after 4 days of storage in the non-coated samples as compared to the coated fresh-cut lotus root samples and continued to increase until 16 days storage. Among all the coated treatments, 0.5% xanthan gum had the highest browning indices among the coated samples during the 16 days of storage at 5°C which suggested that this xanthan gum concentration level was insufficient to create a strong barrier to oxidation and browning.

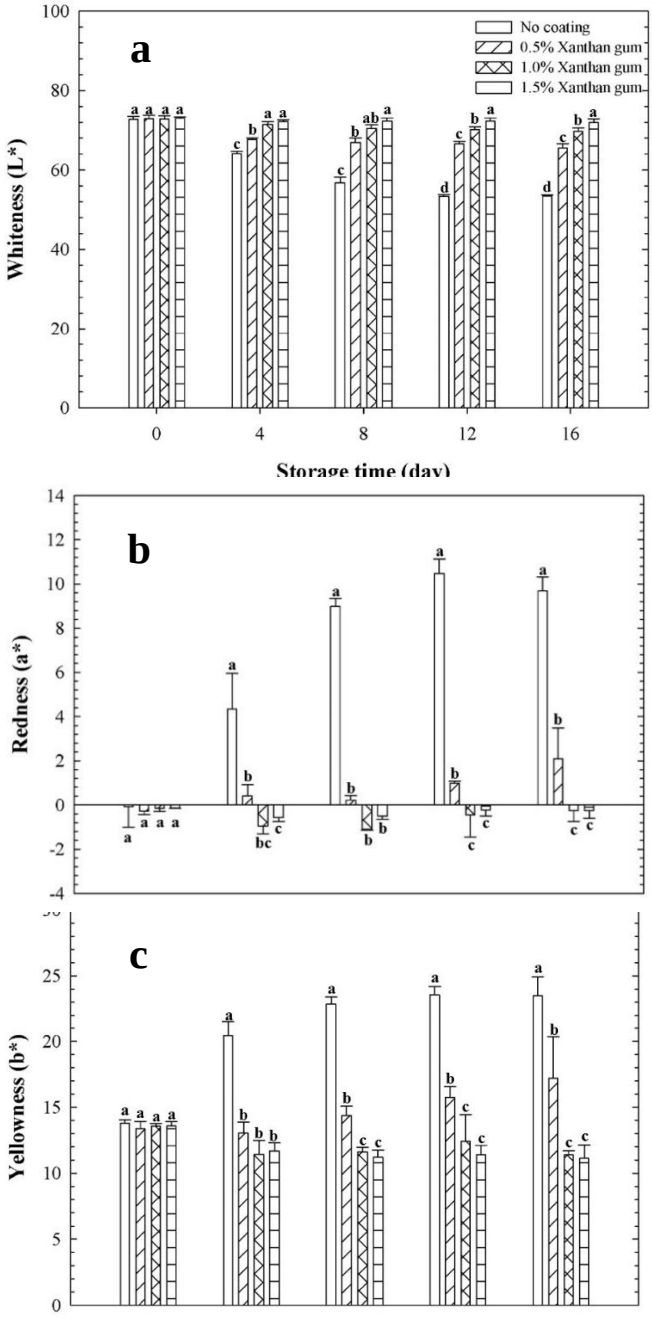


Fig. 3.6 Color changes of fresh-cut lotus root whiteness (a), redness (b) and yellowness (c) during storage at 5 ℃. Different letters indicate significant differences between different treatments at significance level $p < 0.05$.

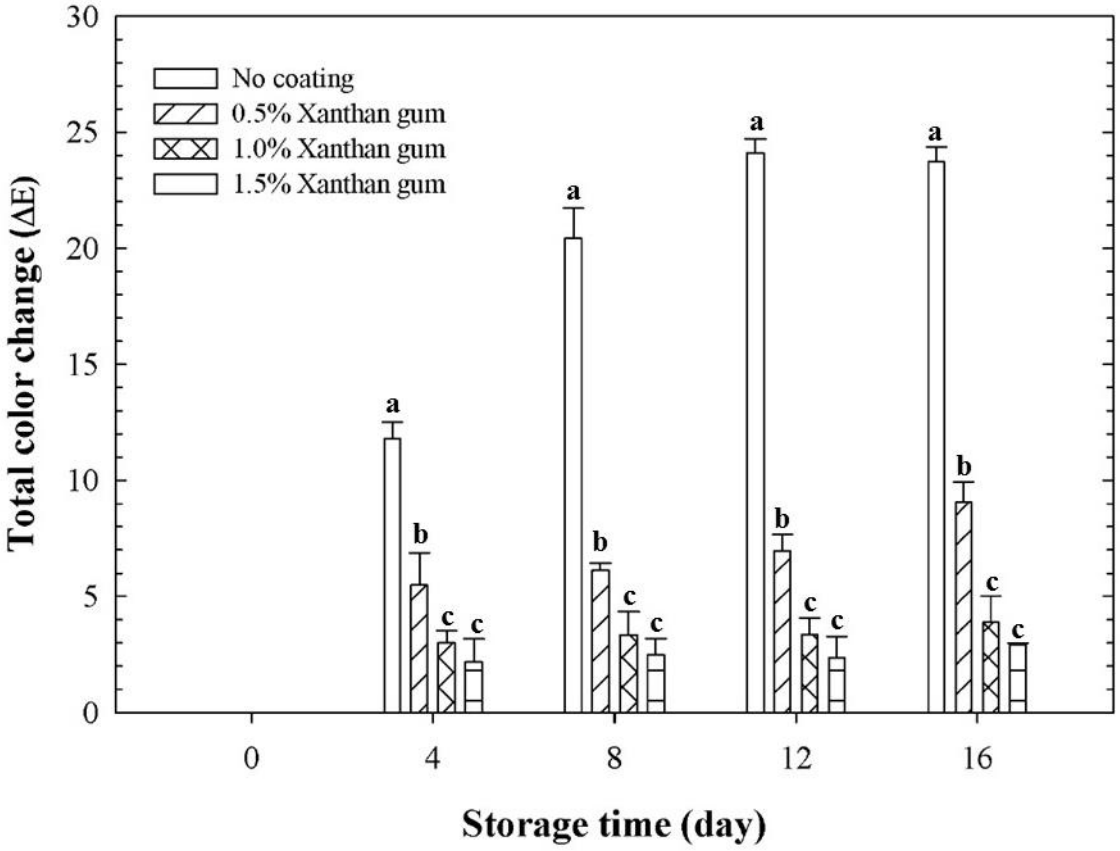


Fig. 3.7 Calculated total color changes of fresh-cut lotus root during storage at 5 °C. Different letters indicate significant differences between different treatments at significance level $p < 0.05$.

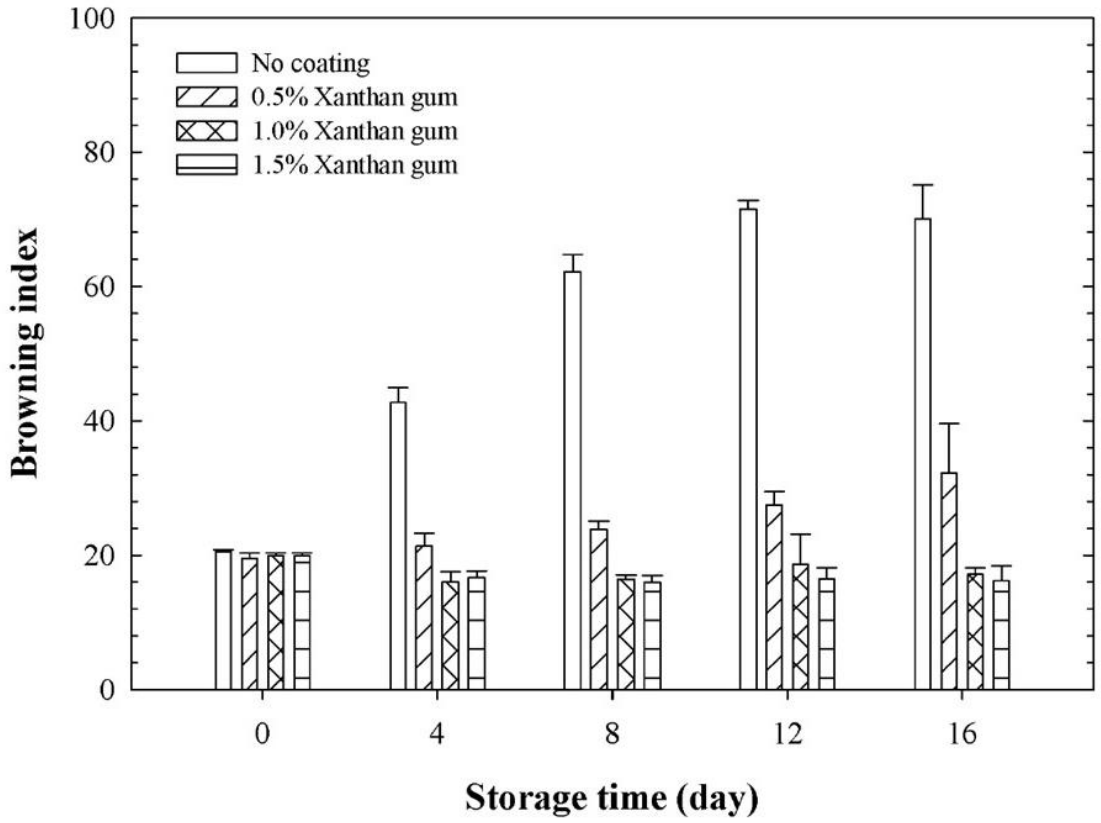


Fig. 3.8 Calculated browning indices of fresh-cut lotus root during storage at 5 ℃. Different letters indicate significant differences between different treatments at significance level $p < 0.05$.

3.3.2.2. Changes in enzymatic activity of Polyphenol Oxidase (PPO) of coated lotus root samples upon storage

Bico et al. (2009) correlated the degree of browning on the lotus root surface with the PPO enzymatic activity. The PPO enzyme plays a significant role in the enzymatic browning process by oxidizing phenolic substrates in the fresh-cut lotus root to form brown pigments (Nguyen et al., 2003). Therefore, the investigation of PPO enzymatic activity and its relation to enzymatic browning is essential in understanding attempts to increase the storage stability of fresh-cut lotus root.

Figure 3.9 presented a significant decrease in the PPO enzymatic activity ($p < 0.05$) for 0.5%, 1.0% and 1.5% xanthan gum and chitosan coated samples during the immediate application of the coating treatments, in comparison with the non-coated samples. The decrease in the PPO enzymatic activity could be attributed to the inactivation of the PPO enzyme upon the application of the coatings wherein a barrier could have possibly been created on the surface of the fresh-cut lotus root. After 4 days of storage, an increase in the PPO activity of the non-coated samples were observed in comparison with the coated samples, which could be correlated to the color changes observed in the non-coated samples during storage. From days 8 to 16, a continuous increase in the PPO enzymatic activities of the non-coated samples were observed while no significant PPO changes ($p < 0.05$) in the coated fresh-cut lotus root samples. Therefore, it was speculated that the application of the xanthan gum and chitosan edible coating created a polymeric barrier that reduced the enzymatic interaction with the atmospheric oxygen and therefore decreased the PPO enzymatic activity. Zhang et al. (2019) reported similar results on the antibrowning effect of xanthan gum coatings encapsulated with star anise essential oil on fresh-cut Chinese yam whereas an 8 times

reduction of browning and PPO activity has been observed in the coated fresh-cut Chinese yam samples as compared to the non-coated samples.

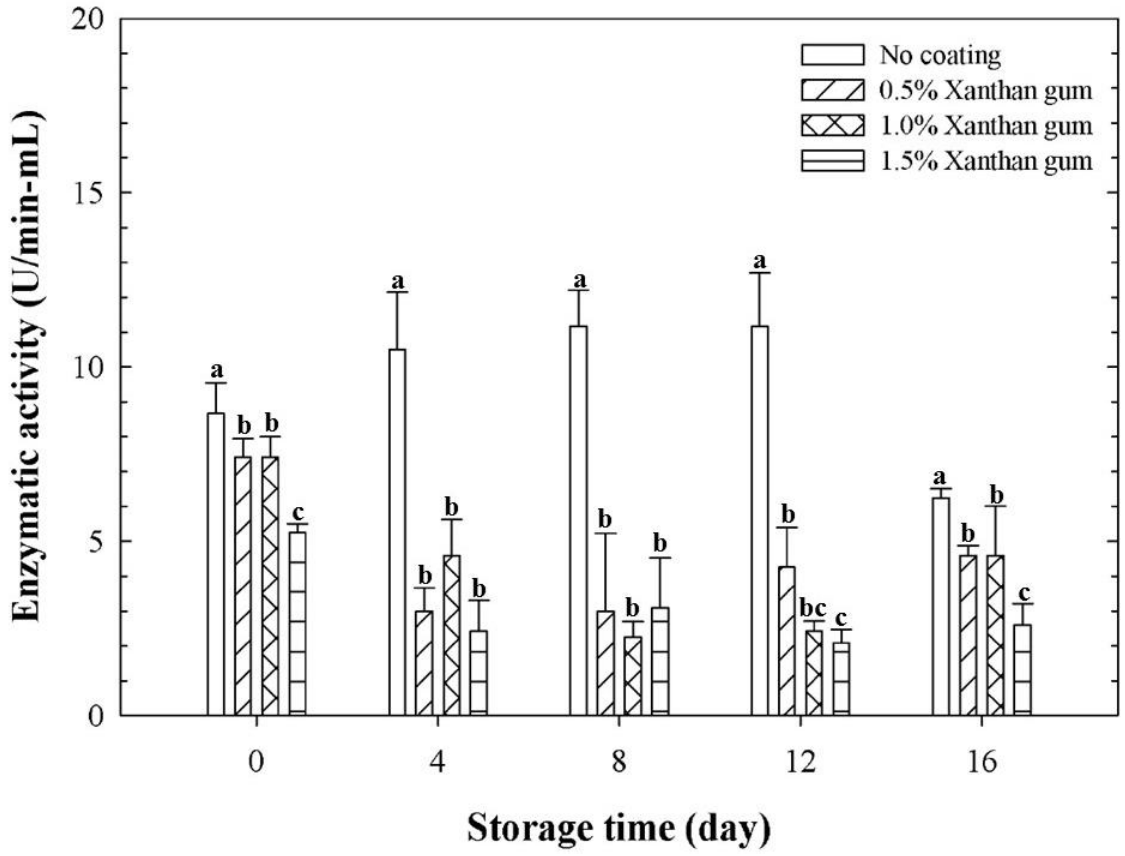


Fig. 3.9 PPO enzymatic activity of fresh-cut lotus root during storage at 5 °C. Different letters indicate significant differences between different treatments at significance level $p < 0.05$.

3.4. Conclusion

In this work, the author featured the improved performance of the layer-by-layer electrostatically deposited xanthan gum-chitosan edible coatings on the fresh-cut lotus root by increasing the xanthan gum concentration in the xanthan gum and chitosan complex. It has been found that an increase in the xanthan gum concentration led to thicker and smoother xanthan gum-chitosan coatings on the surface of the fresh-cut lotus root surface which led to the reduction in total color changes and polyphenol oxidase (PPO) enzyme activity in the coated fresh-cut lotus root samples, in contrast with the non-coated samples. The application of xanthan gum-chitosan edible coatings through the layer-by-layer electrostatic deposition of charges is a simple yet effective technology for increasing the storage stability of fresh-cut lotus root during transport and distribution.

Chapter 4

Spray Technology for Edible Coating Application

4.1. Introduction

Minimal processed fruits and vegetables such as fresh-cut products have been gaining popularity among consumers because of convenience and freshness these products offer, thus the economic significance of the fresh-cut industry is gradually increasing. However, due to physical damage during processing peeling, cutting and slicing, these products have shortened shelf-life and further making these prone to enzymatic browning, texture decay, microbial contamination and undesirable volatile compound production (Khan, et al., 2014; Watada, et al.,1996; Tapia et al., 2008).

An example of a fresh-cut product gaining interest from researchers and industries is the fresh-cut lotus root (*Nelumbo nucifera*). Its white color, crisp texture, pleasant aroma and high nutritional content make this rhizome appealing to consumers (Gao et al., 2017). However, fresh-cut lotus root is prone to enzymatic browning and as a result, deteriorates its quality and shortens its shelf-life. Various strategies from researchers have been reported to improve the shelf-life of the fresh-cut lotus root, mainly focused on chemical methods such as the use of antibrowning and preservatives and physical methods such as the application of gases and modified atmosphere packaging. and Xing et al. (2012) evaluated on the combined effects of antibrowning agents, cinnamon oil fumigation and moderate vacuum packaging while Zhang et al. (2013) confirmed the efficacy of carbon monoxide on browning of fresh-cut lotus root. However, due to the safety concerns of some chemical preservatives and high-costs of equipment for modified atmosphere packaging, one of the promising approaches we had found for improving storage stability of fresh-cut lotus root is the application of edible coatings sourced out from natural gum resources. The edible coating technology is considered to be a feasible,

alternative and promising technology which provides protection, improves appearance, reduces water vapor and air transfer between food product and environment, decreased respiration rates, enzymatic activities and water losses (Silva-Vera et al., 2018). Moreover, edible coatings were reported to reduce respiration, water loss and oxidation rates because of the generation of a modified atmosphere which provides a semi-permeable barrier against gases such as oxygen and carbon dioxide, moisture and solute movement (Ali & Maqbool, 2010).

Various techniques for application of edible coatings to food include dipping, co-acervation and spraying. Several advantages and disadvantages had been reported for each technique and their performance is highly influenced on the characteristics and properties of foods to be coated and the physical properties of the coating (viscosity, density, surface tension). For instance, during the dipping method, the coating suspension could dilute the outer layer of the food surface and could also possibly remove the natural waxy layer of fruits and vegetables which degrade its functionality. Thus, an alternative to these limitations could be the application of edible coatings through the spraying method using spray applicators and air atomizing systems (Silva-Vera et al., 2018). The main advantages of using this technique is the formation of a uniform coating, thickness control and possibility for multilayer applications. In addition, spraying systems prevent the contamination of the coating solution , enable coating solution temperature control and can enhance the automation of continuous production (Andrade et al., 2012).

Therefore, the objective of this research was to evaluate and optimize the operational conditions of a pilot spray system using response surface methodology to achieve an optimum coating on the fresh-cut lotus root (*Nelumbo nucifera*) and its effect

on the physical and barrier properties of xanthan gum-based coatings incorporated with citric acid.

4.2. Materials and methods

4.2.3 Chemicals

Chemical reagents used in this study such as xanthan gum, chitosan, polyvinylpyrrolidone, catechol, sodium phosphate, Folin-Ciocalteu, and gallic acid were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan).

4.2.4. Plant material

The lotus root (*N. nucifera*) samples were purchased from a local supermarket in Tsukuba, Japan. Prior to purchase, the lotus root samples were selected based on whiteness and the length of storage in the supermarket to guarantee that the samples were fresh and of excellent quality for the experiments. The samples were completely washed in running tap water, peeled, and manually sliced into 5 mm thickness using a handheld vegetable cutting machine.

4.2.5. Preparation of spray coating solutions

Xanthan gum solutions were prepared by dissolving 0.1, 0.3, and 0.5 g of powder in 99 mL to achieve 0.1%, 0.3%, and 0.5% concentrations and were magnetically stirred for 2 h at room temperature (HS 360 H, As One Corporation, Tokyo, Japan) to assure the complete dissolution of the powder. After the xanthan gum solutions have been prepared, 2% (w/w) citric acid as an antibrowning agent, and glycerol as a plasticizer were then incorporated in the edible coatings. The selection of glycerol as a plasticizer was based on previous experiments that showed the compatibility of glycerol for xanthan gum-based

coatings. Physico-chemical properties (viscosity and droplet size) and rheological properties (viscosity) were measured before the application of the coatings on fresh-cut lotus root.

4.2.6. Spraying of xanthan gum-based coatings

The coatings were deposited and formed in the fresh-cut lotus root surface using a pilot spray system, described in Fig. 4.1 (EP-Tech, Hitachi Ltd., Tokyo, Japan) to 5 mm thick slices of fresh-cut lotus root for 20 sec at 0.2 MPa, air dried for 1 min, and packed in polyethylene bags, stored for 16 days at 5 °C and analyzed for color, polyphenol oxidase (PPO) enzymatic activity, pH, weight loss, morphology, and microbiological effects against *Bacillus subtilis* spores.

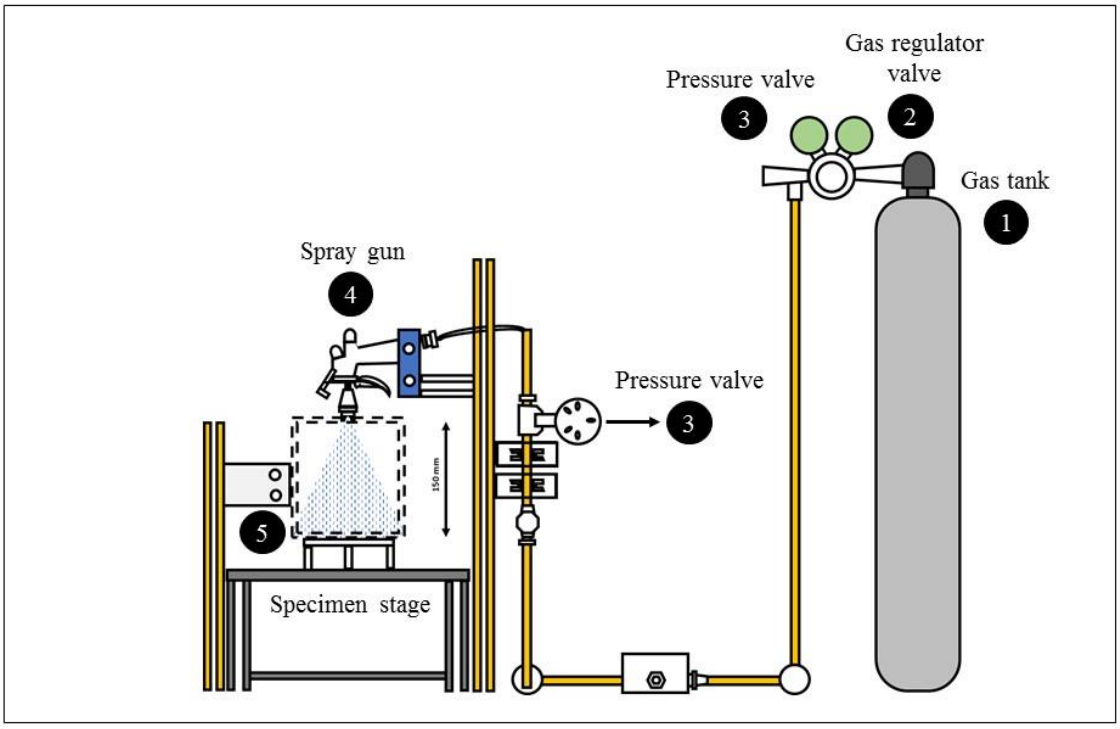


Fig. 4.1 Schematic diagram of the spray coating machine and its parts used in this study.

4.2.7. Analysis of the polysaccharide coating solutions

4.2.7.1. Viscosity

Xanthan gum solutions with concentrations of 0.1% (w/w), 0.3% (w/w), and 0.5% (w/w) were evaluated for viscosity using a Vibro Viscometer (SV-10, A & D Company Ltd., Tokyo, Japan) at 25°C. 35 mL of each coating solutions were placed in the vessel for measurement.

4.2.7.2. Droplet size

Measurements of droplet size were adapted from Zhang et al. (2013). Droplet were collected using a glass slide under the previously mentioned spray process conditions. The collected droplets were then analyzed using an optical and fluorescence microscope (DM-IRM Leica Microsystems, Weizler, Germany).

4.2.7.3. Coating thickness determination

Fresh-cut lotus covered with the spray coatings were stored at 5°C and were analyzed for the coating thickness using an optical and fluorescence microscope (DM-IRM Leica Microsystems, Weizler, Germany). Samples with 0.1 mm cross-sectional cuts were prepared and mounted in a glass slide for thickness measurements.

4.2.7.4. Coating formation on fresh-cut lotus root surface

The formation of the spray coatings on the lotus root samples were detected through the scanning electron microscope (SEM miniscope 1000, Hitachi, Tokyo, Japan). Before the analysis, the non-coated and spray coated lotus root samples were frozen in a normal

deep freezer for 24 h at -18°C and were freeze-dried for 24-48 h (FDU-2100 Eyela Desktop Freeze Dryer, Tokyo, Japan). The freeze-dried samples were then mounted on an aluminum sample probe for magnification at 300x to record SEM images.

4.2.8. Assessment of physico-chemical changes during storage

4.2.8.1. Color

During the 16 days of storage at 5°C, color changes of fresh-cut lotus root were evaluated using spectrophotometer (CM-5, Konica Minolta, Tokyo, Japan). The storage time was kept to 16 days to allow adequate time to assess color and enzymatic changes and detect the stage at which enzymatic browning occur after the application of the coating treatments. Color changes for spray-coated and non-coated samples were expressed using the CIE L*a*b* values obtained from the spectrophotometer. Total color changes (ΔE) were calculated using L*, a*, and b* values, based on the following equation 4.1 reported by Supapvanich (2016) and used previously as:

$$(\Delta E) = [(L_2^*-L_1^*)^2 + (a_2^*-a_1^*)^2 + (b_2^*-b_1^*)^2]^{1/2} \quad (4.1)$$

Browning indices were also calculates using equations 4.2 and 4.3 adapted from Kortei et al. (2015):

$$\text{Browning index (BI)} = \frac{[100-(x-0.31)]}{0.17} \quad (4.2)$$

where:

$$x = \frac{(a^* + 1.75\,L^*)}{5.645\,L^* + a^* - 3.012\,b^*} \quad (4.3)$$

4.2.8.2. pH measurement

Changes in the pH of fresh-cut lotus root were monitored based on Lara et al. (2019) and Son et al. (2015). Fresh-cut lotus root samples (20 g) were homogenized manually with 40 mL of Milli-Q water for 30 s using a mortar and pestle. The pH of the homogenized samples was detected using a digital pH meter (827 pH lab, Metrohm, Herisau, Switzerland).

4.2.8.3. Polyphenol oxidase activity (PPO)

In this study, the enzymatic activity changes of polyphenol oxidase (PPO) in the non-coated and spray-coated lotus root samples were evaluated based on the methodology adapted from Lara et al. (2019) and Son et al. (2015). Fresh-cut lotus root samples (10 g) were homogenized manually using a mortar and pestle with sodium phosphate buffer (0.2 mol/L, pH 7.0 with 2% polyvinylpyrrolidone) following a ratio of 2.0 mL of buffer per 1 g of lotus root in an ice bath. The homogenates were centrifuged (high-speed refrigerated centrifuge, Tomy MX -307, Tomy Seiko, Tokyo, Japan) at 12000 × *g* for 10 min at 4 °C. The supernatant, which was used further for analysis, was separated from the filtrate by decanting the liquid into another centrifuge tube. The enzymatic activity was determined by the increase of absorbance at 410 nm for catechol at 25 °C using a spectrophotometer (UV-Vis, V-530, JASCO Inc., Tokyo, Japan). The 0.2 mL supernatant was added with 2.8 mL of the catechol substrate solution (0.02 mol/L catechol in 0.05 mol/L sodium phosphate buffer, pH 7). The catechol substrate solution (catechol in sodium phosphate buffer) was used as the reference. The enzymatic activity (units/(min-mL enzyme)) was detected by the linear section of the activity curve. The 1 unit of PPO enzyme activity was defined as the change in 0.001 in the absorbance value per min.

4.2.8.4. Weight loss

The weight loss from samples was also monitored during the 16 days of storage at 5°C. The values were determined and expressed as percent losses from the initial weights of the sample using Eq. 4.4, as reported previously as:

$$\% WL = \frac{w_0 - w_d}{w_0} \times 100 \qquad (4.4)$$

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

4.2.9. Microbiological analysis

For the microbiological analysis, 0.1%, 0.3%, and 0.5% xanthan gum-based coatings were sprayed for 10 and 20 s on plate count agar (PCA) plates inoculated with *Bacillus subtilis* 1465 spores. The plates were incubated for 24-48 h at 37°C (Biomatic Digital Microscope Culturing System FA-100, Tokyo, Japan). *B. subtilis* colonies were counted manually and results were expressed as CFU/mL (colony forming units per mL of spray coating solution).

4.2.10. 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 viscosity, pH, texture, color, weight loss, and enzymatic activity. 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.

4.3. Results and discussions

4.3.1. Characteristics of the polysaccharide-based coatings for fresh-cut lotus root application

The physicochemical (droplet size and coating thickness) and rheological properties (viscosity) of the coating solutions for spraying were evaluated before fresh-cut lotus root application (Table 4.1). Initially, effect of increasing xanthan gum concentration on the viscosity of the coating solutions were determined. Based on our results, we observed that an increase in the xanthan gum concentration from 0.1% (w/w) to 0.5% (w/w), resulted to an increase in the viscosities of the solutions from 6.04 mPa.s to 75.00 mPa.s. Furthermore, the increased viscosities led to an increase of coating thickness from 17.03 μm to 66.67 μm as clearly seen in Fig. 4.2. Zevallos & Krochta (2003) obtained similar results wherein an increase in the hydroxypropyl methylcellulose (HPMC) coating viscosity led to an increase of coating thickness in fresh-cut apple slices. On the other hand, droplet sizes of the spray coatings were also measured for the first time using a hydrophobic glass slide (Fig. 4.3). Based on our results, the average droplet sizes were between 5.14 to 5.61 μm. Zhang et al. (2013) reported that droplets from electrospraying had sizes between 2 μm.

Table 4.1 Physico-chemical properties of the coating treatments used in the study.

Xanthan gum [%,w/w]	Viscosity [mPa.s]	Droplet size [μm]	Coating thickness [μm]
0.10	6.04 ± 0.01	5.61 ± 0.75	17.03 ± 1.30
0.30	36.53 ± 0.25	5.14 ± 0.75	18.33 ± 0.00
0.50	75.00 ± 0.60	5.14 ± 1.28	66.67 ± 15.28

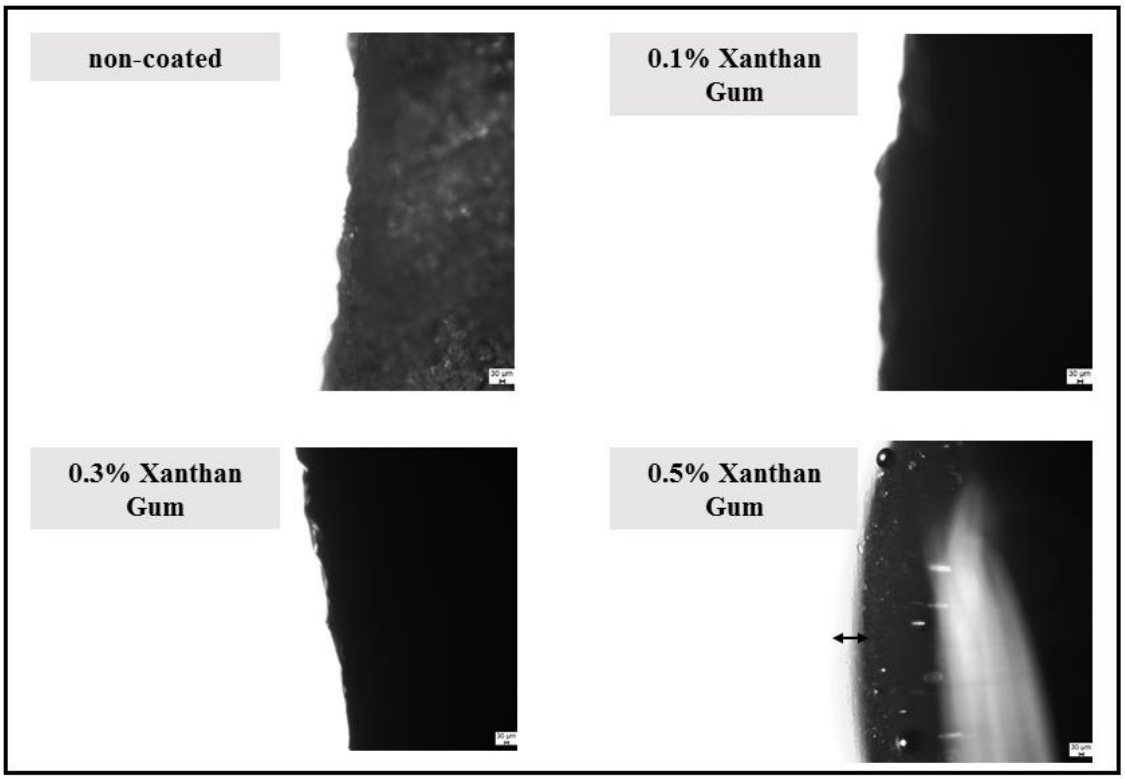


Fig. 4.2 Optical microscopy measurements for the thickness of the spray coatings in fresh-cut lotus root.

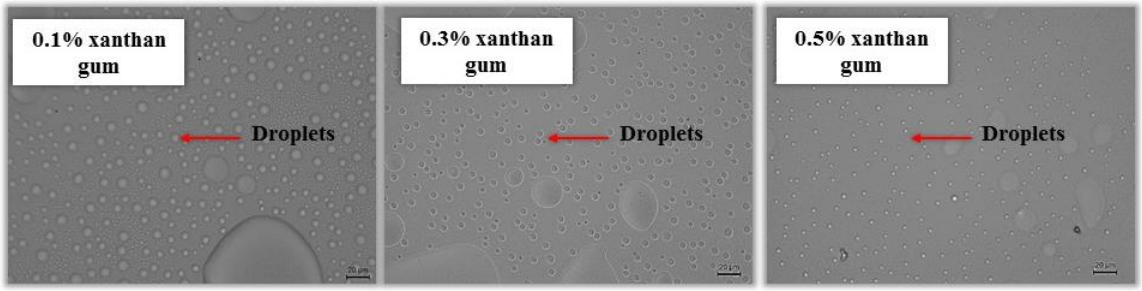


Fig. 4.3 Droplet size measurements of the spray coatings.

4.3.1.1. Evaluation of coating formation on lotus root surface through scanning electron microscopy (SEM)

In this work, the formation of the spray coatings on the fresh-cut lotus root during day 0 was highlighted (Fig. 4.4). Initial micrographs of fresh-cut lotus root without any treatment demonstrated a clear image of the cell wall and numerous spherical shaped parts which signified the presence of starch granules. The absence of a coating barrier in the lotus root surface exposed the cell structures of the lotus root which may lead to oxidation of the lotus root samples during storage. Contrastingly, the application of 0.1%, 0.3%, and 0.5% spray coatings on fresh-cut lotus root surface resulted to a uniform distribution of spray droplets. Based on this study, increasing the xanthan gum concentration from 0.1% to 0.3%, and 0.5% formed smoother coating layer on the surface of fresh-cut lotus root. However, despite the smooth surface in the lotus root samples, distinct cracks were observed for 0.1%, 0.3%, and 0.5% spray coated samples, which may reduce the barrier properties of the spray coatings against oxidation and moisture migration. Khan et al. (2014) accounted that “zwarwit” candy tablets coated with chocolate coatings by electrospraying resulted to pinhole formation on the surface of the chocolate-based coatings which remained and became larger during 6 days of storage. However, based on their results, the inner coatings were not damaged throughout the storage, and hence the coatings storage stability was not affected.

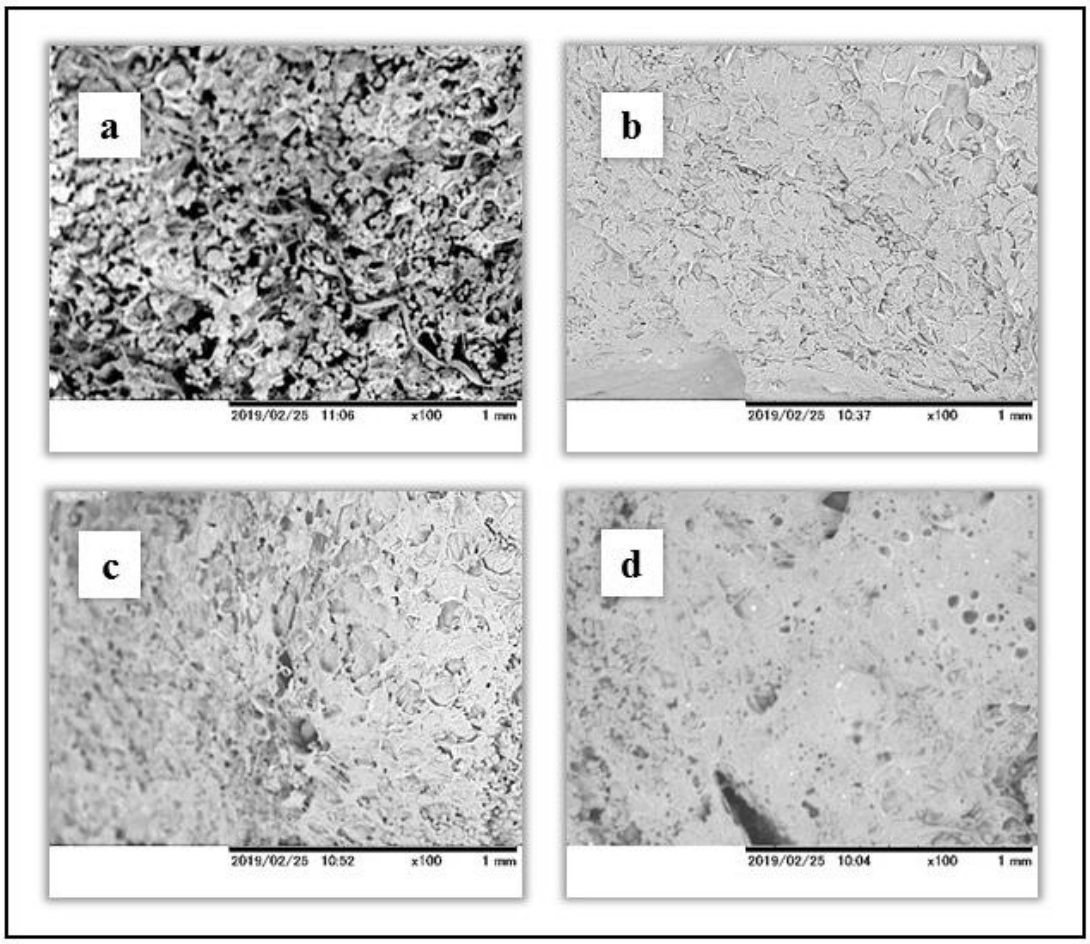


Fig. 4.4 SEM micrographs of edible coatings on the surface of fresh-cut lotus root at day 0 for: (a) control; (b) xanthan gum; (c) guar gum; (d) chitosan; (e) xanthan gum + chitosan; (f) guar gum + chitosan

4.3.2. Assessment of physico-chemical changes during storage

4.3.2.1. Changes in color of spray-coated lotus root during storage

The visual quality of fresh-cut products is one of the most significant attributes that influence that decisiveness of consumers in purchasing products in the market (Cakmak et al., 2018). Hence, the investigation of color changes of fresh-cut lotus root during storage is a critical consideration during the application of edible coatings.

Figure 4.5 showed that color changes (whiteness, redness, and yellowness) of fresh-cut lotus root during 16 days of storage at 5°C. As can be observed from the results, the whiteness values (L*) of non-coated samples started to decrease sharply after 4 days and throughout the storage time, as compared to spray-coated samples. These results were evident in Fig. 4.5a wherein the whiteness values of non-coated samples decreased sharply from the initial value of 72.32 to 42.68. Son et al. (2015) reported similar results wherein fresh-cut lotus root without any treatment has increased browning rates as compared to samples treated with antibrowning agents and modified atmosphere packaging (MAP). This could be attributed to the absence of coatings layer systems in the non-coated samples, which may act as a barrier to gases such as oxygen and carbon dioxide (Valdés et al., 2017). Meanwhile, the whiteness values of the spray-coated samples were significantly maintained ($p < 0.05$), however, it started to decrease after 12 days of storage. The decrease of whiteness values in spray-coated samples could be explained by the possible diffusion of the edible coatings inside the fresh-cut lotus root and thus, the exposure of the lotus sample surface to oxidation. On the other hand, the increase of redness (a*) and yellowness (b*) values were also observed in the coated and non-coated samples during the 16 days storage which could also be related to the

browning phenomena in the samples, as presented in Fig. 4.5b and 4.5c. However, based on our results, the application of the spray coating on the fresh-cut lotus root samples significantly reduced the redness and yellowness color changes in the samples ($p < 0.05$) which could signify the efficacy of the spray coating treatments in extending the storage stability of fresh-cut lotus root. Furthermore, with the use of the reported values of L*, a*, and b*, the total color changes (ΔE) and browning index were calculated and presented in Fig. 4.6 and Fig. 4.7. It was observed that the application of spray coatings at 0.1%, 0.3%, and 0.5% in fresh-cut lotus root significantly reduced color changes ($p < 0.05$) up to 60.96%, 74.12%, and 84.38% respectively. These results evidently proved that the spray coatings of xanthan gum and citric acid were effective in reducing oxidation and an increase of the xanthan gum concentration could increase the barrier layer in the fresh-cut lotus root surface, signified as thickness, thus improving its functionality in extending the storage stability of fresh-cut lotus root. Among all the spray coating treatments, 0.5% of xanthan gum had the highest reduction of total color changes which exemplified that an increase in xanthan gum concentration could possibly lead to increase oxidation barrier functionality. The calculated browning indices reported in Fig. 4.7 were also in accordance with these results, which suggested that significant browning ($p < 0.05$) occurred in non-coated samples as compared to spray-coated samples during 16 days of storage. The application of spray coatings in the fresh-cut lotus root samples led to 71.15%, 69.27%, and 69.27% significant reductions of browning indices in 0.1%, 0.3%, and 0.5% spray coated samples respectively.

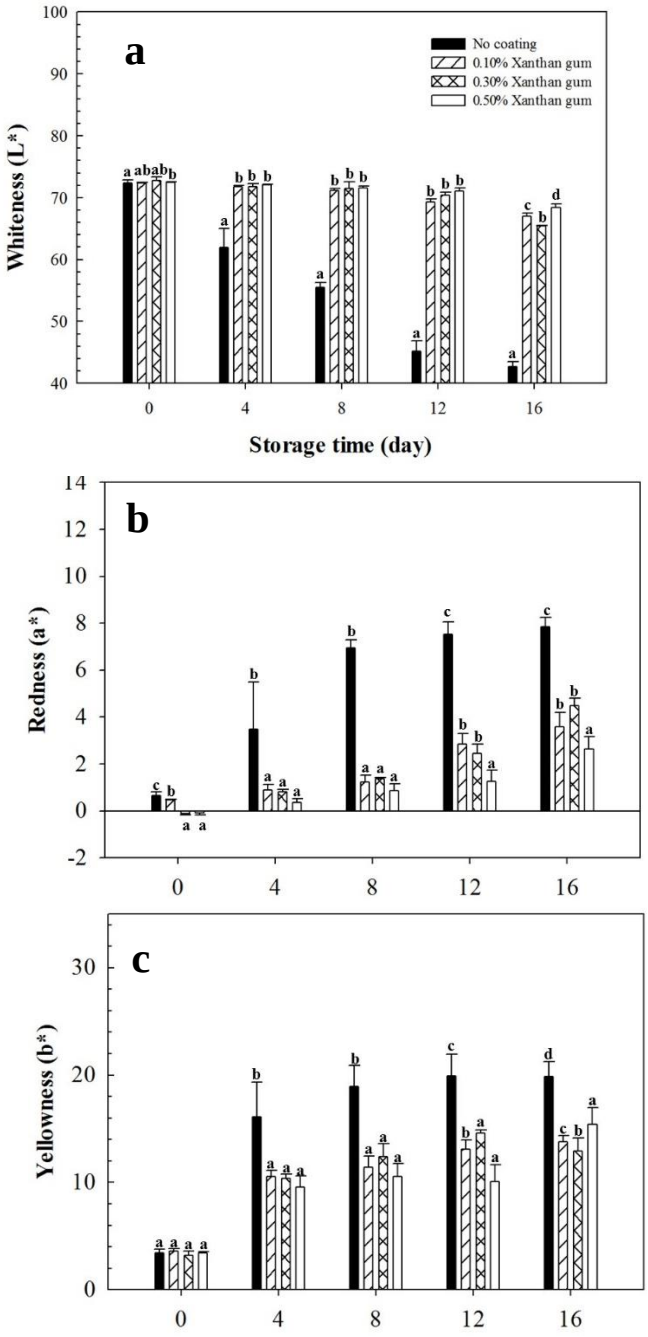


Fig. 4.5 Color changes of fresh-cut lotus root during storage at 5 °C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

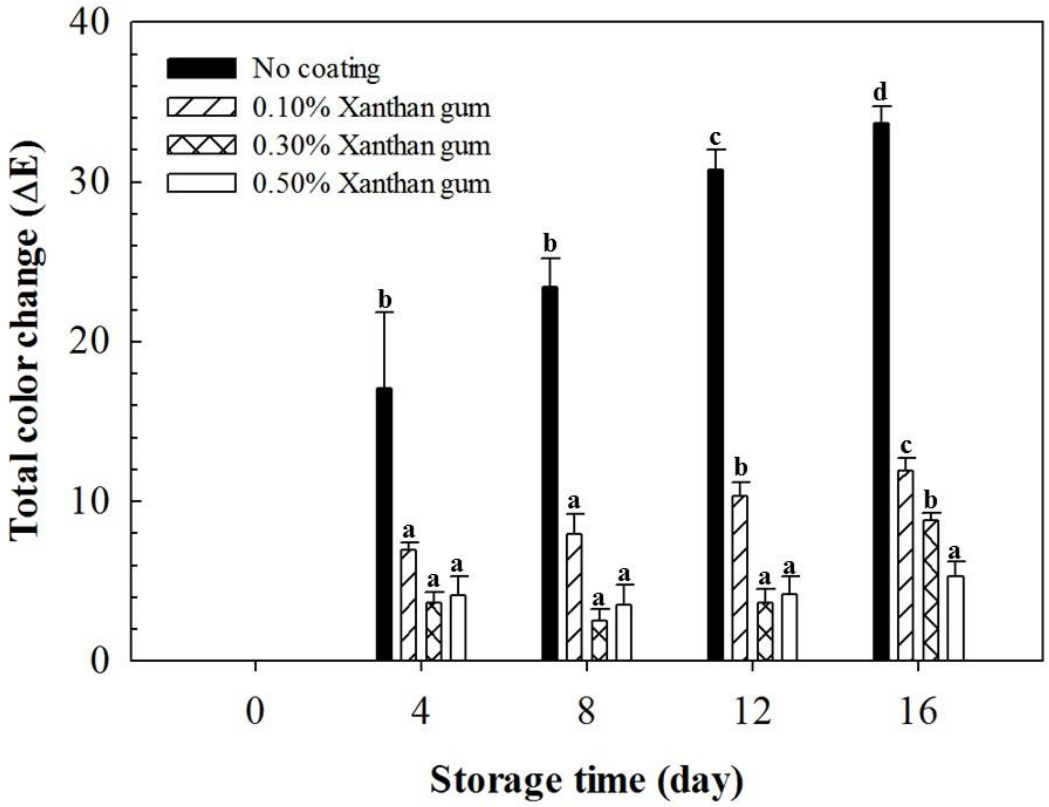


Fig. 4.6 Total color changes of fresh-cut lotus root during storage at 5 °C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

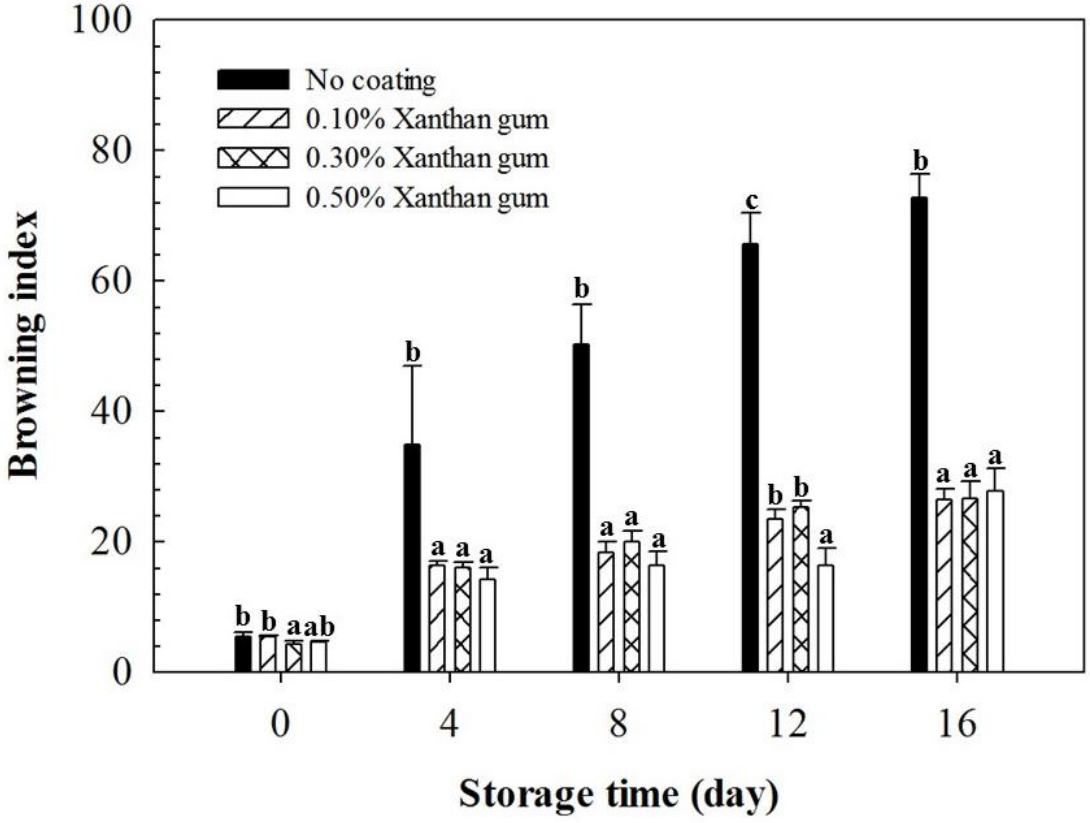


Fig. 4.7 Browning indices of fresh-cut lotus root during storage at 5 °C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

4.3.2.2. Changes in Polyphenol Oxidase (PPO) enzymatic activity of spray-coated lotus root during storage

Bico et al. (2009) reported that the degree of enzymatic browning on the lotus root surface is highly correlated with the PPO enzymatic activity. The PPO enzyme results to enzymatic browning by the oxidation of phenolic compounds in the fresh-cut lotus root which further forms brown pigments (Nguyen et al., 2003). It is therefore vital to assess enzymatic activity and its relation to enzymatic browning in order to increase the stability of fresh-cut lotus root during storage. During storage, the application of 0.1%, 0.3%, and 0.5% xanthan gum spray coatings on fresh-cut lotus root decreased significantly the PPO enzymatic activity (Fig. 4.8). The decrease in the PPO enzymatic activity could be attributed to the possible creation of a semi-permeable barrier layer in the surface of the fresh-cut lotus root, hence, prevented the direct contact of atmospheric oxygen with the fresh-cut lotus root samples, therefore, reducing the browning enzyme's activity. After 4 days of storage, PPO enzymatic activity of the non-coated samples increased in comparison with the spray-coated samples. This enzymatic increase could be associated with the color changes associated with browning in the non-coated samples during storage. Meanwhile, significant reductions of PPO enzymatic activity up to 87%, 71.83%, and 63.20% were observed for 0.1%, 0.3%, and 0.5% spray coating xanthan gum treatments respectively. From 8 to 16 days of storage, PPO enzymatic activities of all spray coated samples were significantly lower ($p < 0.05$) as compared to non-coated samples. Based on our results, we speculated that the application of spray coatings of xanthan gum and citric acid generated a polymeric barrier that reduced the enzymatic interaction with the atmospheric oxygen, and therefore, reduced the PPO enzymatic activity. Sharma & Rao (2015) obtained similar results whereby xanthan gum enriched

with cinnamic acid (1 g/L) resulted to significant retardation of oxidative browning ($p < 0.05$), reduction of ascorbic acid level and degradation of total phenolics in fresh-cut pears, in comparison with non-coated and xanthan gum only coated fresh-cut pears.

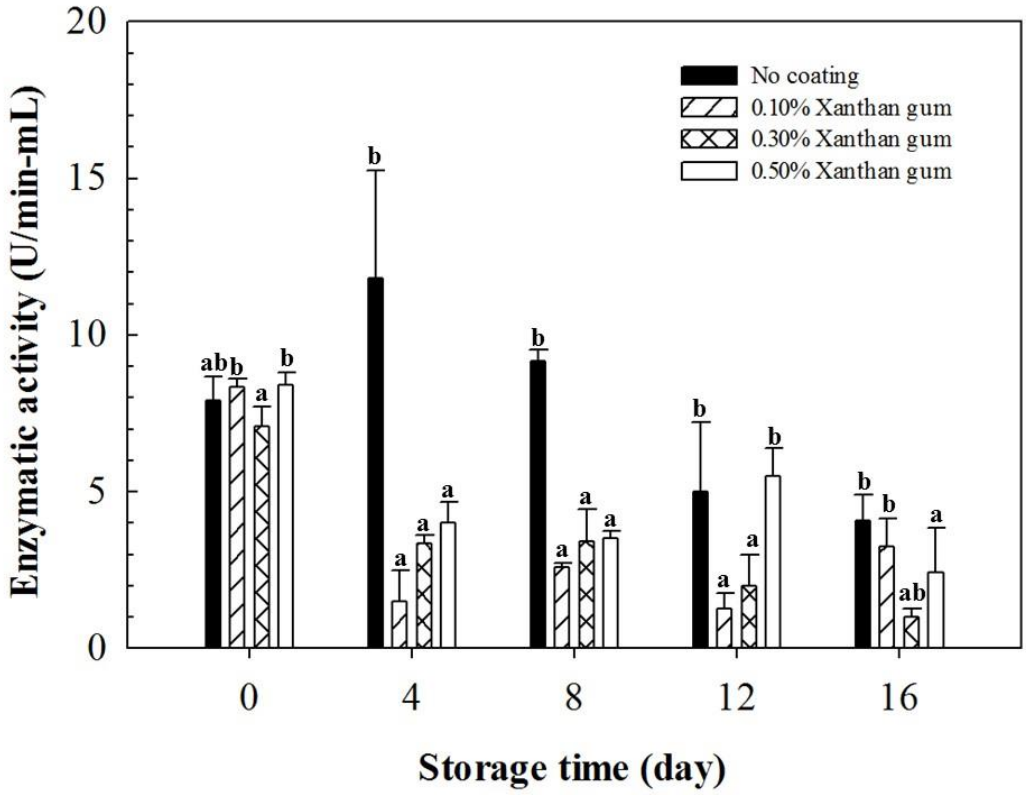


Fig. 4.8 PPO enzymatic activity of fresh-cut lotus root during storage at 5 °C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

4.3.2.3. Changes in weight loss of spray-coated lotus root samples during storage

Weight loss in fresh-cut fruits and vegetables is associated to respiration, transpiration, and loss of water due to minimal processing such as peeling and slicing (Zhang et al., 2019). As presented in Fig. 4.9, weight loss of fresh-cut lotus root samples increased with 16 days storage time, regardless of the treatments. However, we found out that weight loss of spray-coated samples were lower than that of the non-coated samples after 4 days of storage time. The weight loss reduction in spray coated samples could be attributed to the effect of the coatings as a semi-permeable barrier against O_2, CO_2, moisture, and solute movement consequently reducing respiration, water loss, and oxidation reaction rates (Ali et al., 2010). Similar results were reported by Sharma (2019) wherein bilayer coatings of native/crosslinked sesame protein guar gum and mango puree resulted to reduced weight loss of fresh-cut mangos during 15 days of storage. The same trend of reduced weight in spray-coated lotus root samples were observed from 8 to 16 days of storage. However, we speculate that despite the weight loss reductions of xanthan gum spray coatings on fresh-cut lotus root, the incorporation of citric acid, which lowered the pH of the spray coatings, contributed to the elevated weight loss percentages in spray-coated lotus root samples. Previously, we reported the same results in which the application of acidic treatments in fresh-cut lotus root contributed to elevated weight loss percentages, which could be attributed to the microstructure damage of lotus root, consequently, causing tissue softening and weight loss (Lara et al., 2019).

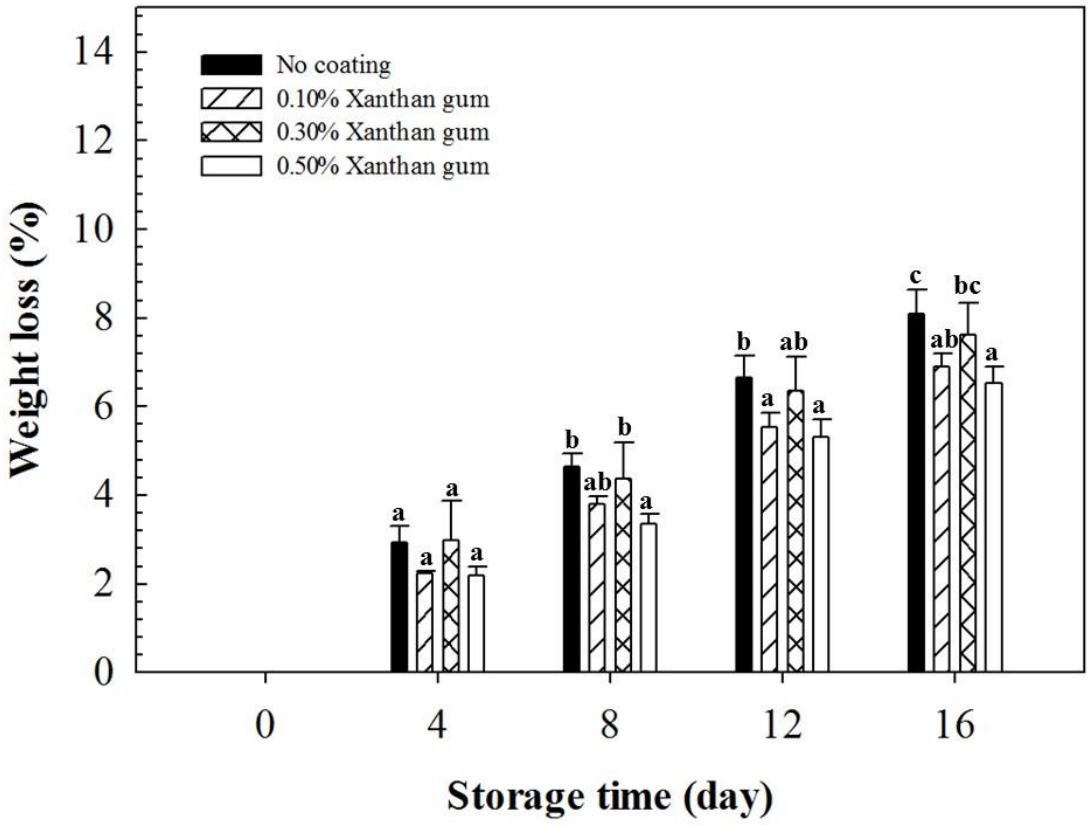


Fig. 4.9 Weight loss changes of fresh-cut lotus root during storage at 5 °C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

4.3.2.4. Changes in pH of spray-coated samples during storage

Differences in the pH values of spray-coated and non-coated lotus root samples during the 16 days of storage were also monitored (Fig. 4.10). Based on our results, an increase of pH in spray coated and non-coated lotus root samples were observed during the 16-day storage. This increase in pH could be attributed to the breakdown of the major components of lotus root such as 13% protein, 2.3% crude fat, 66% carbohydrates, and 2.8% fiber (Lara et al., 2019). Mannozzi et al. (2017) reported similar results of sodium alginate and pectin-based edible coatings wherein an increase of pH and soluble solid content (SSC) were observed in blueberry fruits during 14 days of storage. The increase of pH and SSC during storage could be related to the metabolic processes and interactions during post-harvest storage such as the conversions of starch and acids into sugars. The initial pH of fresh-cut lotus root was 6.53, however, with the application of the spray coatings, the pH was reduced to 5.06, 4.73, and 4.81 for 0.1%, 0.3%, and 0.5% xanthan gum spray coatings respectively. The reduction of pH in spray coated lotus root samples were maintained until 12 days of storage and were observed to increase to 6.57, 4.84, and 6.49 0.1%, 0.3%, and 0.5% xanthan gum spray coatings respectively after 16 days of storage. We speculate that the increase of pH in the spray coated and non-coated samples could possibly explain the occurrence of enzymatic browning during storage whereby the observed pH has increased towards the optimal pH range for PPO which was pH 4.0-8.0 (Yoruk & Marshall, 2003).

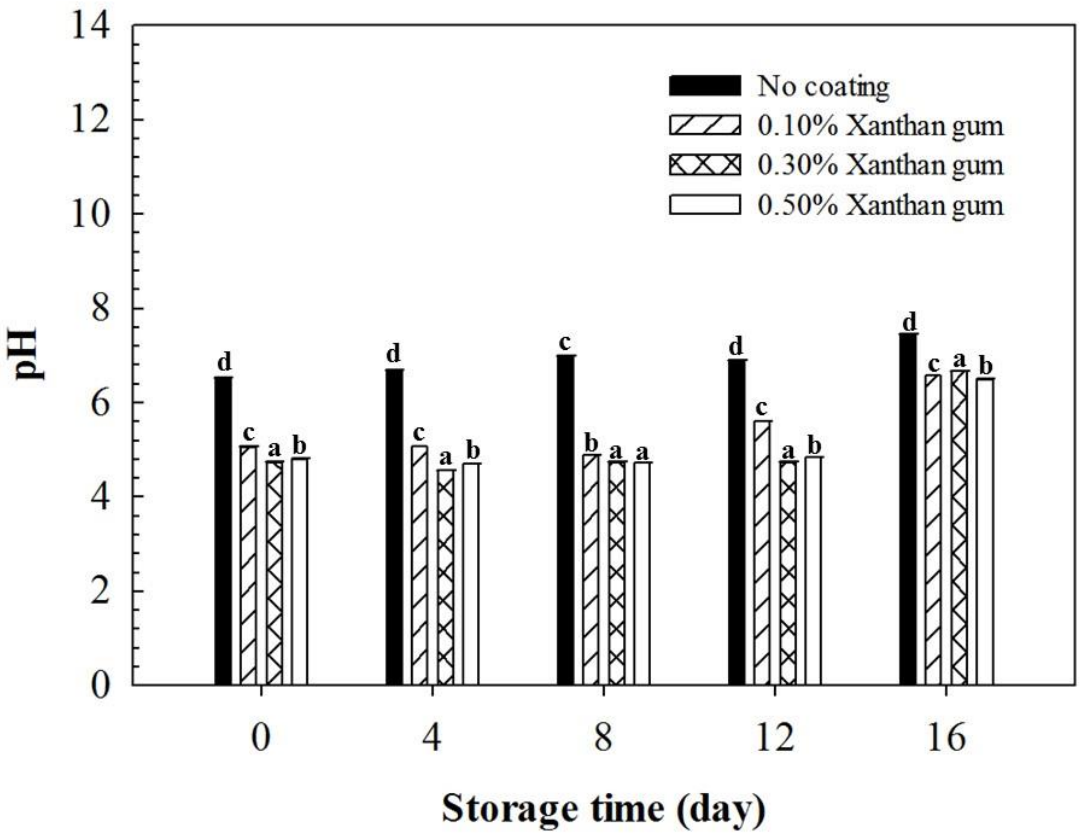
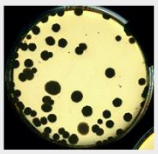
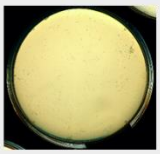
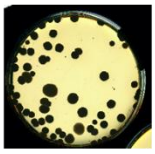
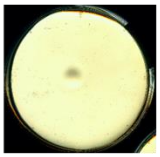
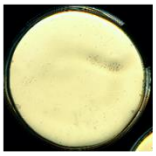


Fig. 4.10 pH changes of fresh-cut lotus root during storage during storage at 5 °C. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

4.3.3. Evaluation of microbiological effects of spray coating treatments on *Bacillus subtilis*

Microbial quality is one of the significant parameters needed to be assessed for the effective preservation of fresh-cut produce (Bico et al., 2009). Figure 4.11 shows the effect of the 0.1%, 0.3%, and 0.5% xanthan gum spray coatings and the duration of spraying time (10 s and 20 s) on the viability of gram-positive strains of *Bacillus subtilis.* Based on the obtained results, non-sprayed samples exhibited *B. subtilis* counts of 36 X 10^7 CFU/mL while 0 CFU/mL were recorded for all coating treatments (0.1%, 0.3%, and 0.5%) upon spraying for 20 s. Park et al. (2017) reported the similar results wherein antimicrobial spray nanocoating based on Fe (III) tannic acid enhanced the shelf life of mandarin and strawberries by preventing microbial growth in these food products. Meanwhile, Peretto et al. (2017) also accounted that alginate coatings which were electrostatically and non-electrostatically sprayed on strawberries reduced fungal decay during 13 days of storage. On the other hand, it was observed that reducing the spraying time to 10 s decreased the antimicrobial efficacy of the 0.1%, 0.3%, and 0.5% spray coatings against *B. subtilis*. With the application of spray coatings in 10 s only, the *B. subtilis* counts increased to 3.6 X 10^8, 4.33 X 10^5, and 8.00 X 10^5 for 0.1%, 0.3%, and 0.5% spray coatings respectively. Based on these results, it can be speculated that the duration of spraying process influenced the efficacy of spray coatings in inhibiting the growth of *B. subtilis*.

Spraying time (seconds)	No spray coating	0.1% XG* + CA**	0.3% XG + CA	0.5% XG + CA
10				
20				

*XG: Xanthan gum **CA: 2% (w/w) Citric acid

Fig. 4.11 Microbial analysis of *Bacillus subtilis* after application of spray coatings after 24 h incubation at 35 ° C.

4.4. Conclusions

In this part of the work, it has been found that spray-coated fresh-cut lotus root samples had significant reduction in the total color changes as compared to non-coated samples. These results suggested that the spray coating treatments were effective in decreasing the enzymatic browning of fresh-cut lotus root during storage which could potentially increase its shelf-life in the market. In addition, the author also found that the xanthan gum-based spray coated treatments were also effective against inhibiting the growth of *Bacillus subtilis* during 24 h of incubation which were indicated by the lower microbial counts recorded as compared to non-coated fresh-cut lotus root samples. In this part of the work, the author highlighted the spray coating technique of xanthan gum-based edible coatings as a promising strategy in improving the storage stability of fresh-cut lotus root during post-harvest storage.

Chapter 5

Comparative studies on dipping and spraying application of edible coatings

5.1 Introduction

One of the alternative and promising approaches for the shelf life extension of fresh-cut lotus root is the application of edible coatings (Chapter 1). These coatings were reported to provide a semi-permeable barrier to gases and water vapor which reduced respiration rates, enzymatic browning, and water loss. Due to the limited information on the application of edible coating systems on fresh-cut lotus root, the author developed an effective and low cost edible coatings from natural sources such as gums and marine polysaccharides and evaluated its effects on quality changes of fresh-cut lotus root such as color, texture, enzymatic activity, total phenols, and microstructural changes during 16 days of storage at 5°C.

Various techniques for application of edible coatings to food include dipping, co-acervation and spraying. Several advantages and disadvantages had been reported for each technique and their performance is highly influenced on the characteristics and properties of foods to be coated and the physical properties of the coating (viscosity, density, surface tension).

In Chapter 4, the author evaluated the effect of the spraying method as an application technique for xanthan gum-based edible coatings and investigated its barrier and microbial properties on fresh-cut lotus root. Xanthan gum solutions (0.1%, 0.3%, and 0.5%) were prepared, magnetically stirred for 2 h at room temperature and incorporated with citric acid as an antibrowning agent and 1% (w/w) glycerol as plasticizer. The coatings were sprayed using a pilot spray system to 5 mm thick slices of fresh-cut lotus root for 20 s, packed in polyethylene bags, stored for 16 days at 5°C and analyzed for color, pH, morphology and microbial counts. It was found that spray-coated fresh-cut

lotus root samples had significant reduction in the total color changes as compared to non-coated samples. These results suggest that the spray coating treatments were effective in decreasing the enzymatic browning of fresh-cut lotus root during storage which could potentially increase its shelf-life in the market. In addition, the author also found that the xanthan gum-based spray coated treatments were also effective against inhibiting the growth of *Bacillus subtilis* during 24 h of incubation which were indicated by the lower microbial counts recorded as compared to non-coated fresh-cut lotus root samples. In this part of the work, the author highlighted the spray coating technique of xanthan gum-based edible coatings as a promising strategy in improving the storage stability of fresh-cut lotus root during post-harvest storage.

In this chapter, the author evaluated the differences of dipping and spraying methods in terms of reducing the color changes of fresh-cut lotus root during storage.

5.2 Materials and methods

5.2.1 Plant material

The lotus root (*N. nucifera*) samples were purchased from a local supermarket in Tsukuba, Japan during the September to December. Prior to purchase, the lotus root samples were selected based on whiteness and the length of storage in the supermarket to guarantee that the samples were fresh and of excellent quality for the experiments. The samples were completely washed in running tap water, peeled, and manually sliced into 5 mm thickness using a handheld vegetable cutting machine.

5.2.2 Preparation of dipping and spray coating solutions

Xanthan gum solutions were prepared by dissolving 0.1, 0.3, and 0.5 g of powder in 99 mL to achieve 0.1%, 0.3%, and 0.5% concentrations and were magnetically stirred for 2 h at room temperature (HS 360 H, As One Corporation, Tokyo, Japan) to assure the complete dissolution of the powder. After the xanthan gum solutions have been prepared, 2% (w/w) citric acid as an antibrowning agent, and glycerol as a plasticizer were then incorporated in the edible coatings. The selection of glycerol as a plasticizer was based on previous experiments that showed the compatibility of glycerol for xanthan gum-based coatings. Physico-chemical properties (viscosity and droplet size) and rheological properties (viscosity) were measured before the application of the coatings on fresh-cut lotus root and were reported in Chapter 4.

5.2.3 Dipping of xanthan gum-based coatings

Five slices (5 mm thick) of fresh-cut lotus root per treatment were immersed for 2 min in the xanthan gum coating solutions (Fig. 5.1). Preliminary studies were done prior to this experiment on the dipping time. There were only slight differences on storage of fresh-cut lotus root if the dipping time is extended further. The fresh-cut lotus root samples were then quickly dried naturally by letting the excess citric acid drip off from the surface of the samples. The fresh-cut lotus root slices were packed in polyethylene bags (60 μm x160 mm x 260 mm; GT-1626, Sigma Tube, Tokyo, Japan) and were stored for 16 days at 5 ℃ for to evaluate color parameters.

5.2.4 Spraying of xanthan gum-based coatings

The coatings were deposited and formed in the fresh-cut lotus root surface using a pilot spray system, described in Fig. 5.2. (EP-Tech, Hitachi Ltd., Tokyo, Japan) to 5 mm thick slices of fresh-cut lotus root for 20 sec at 0.2 MPa, air dried for 1 min, and packed in polyethylene bags, stored for 16 days at 5 °C and analyzed for color.

5.2.5 Assessment of color

During the 16 days of storage at 5°C, color changes of fresh-cut lotus root were evaluated using spectrophotometer (CM-5, Konica Minolta, Tokyo, Japan). The storage time was kept to 16 days to allow adequate time to assess color and enzymatic changes and detect the stage at which enzymatic browning occur after the application of the coating treatments. Color changes for spray-coated and non-coated samples were

expressed using the CIE L*a*b* values obtained from the spectrophotometer. Total color changes (ΔE) were calculated using L*, a*, and b* values, based equation 5.1 reported by Supapvanich (2016) and used previously:

$$(\Delta E) = [(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2]^{1/2} \quad (5.1)$$

5.2.6 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. 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.

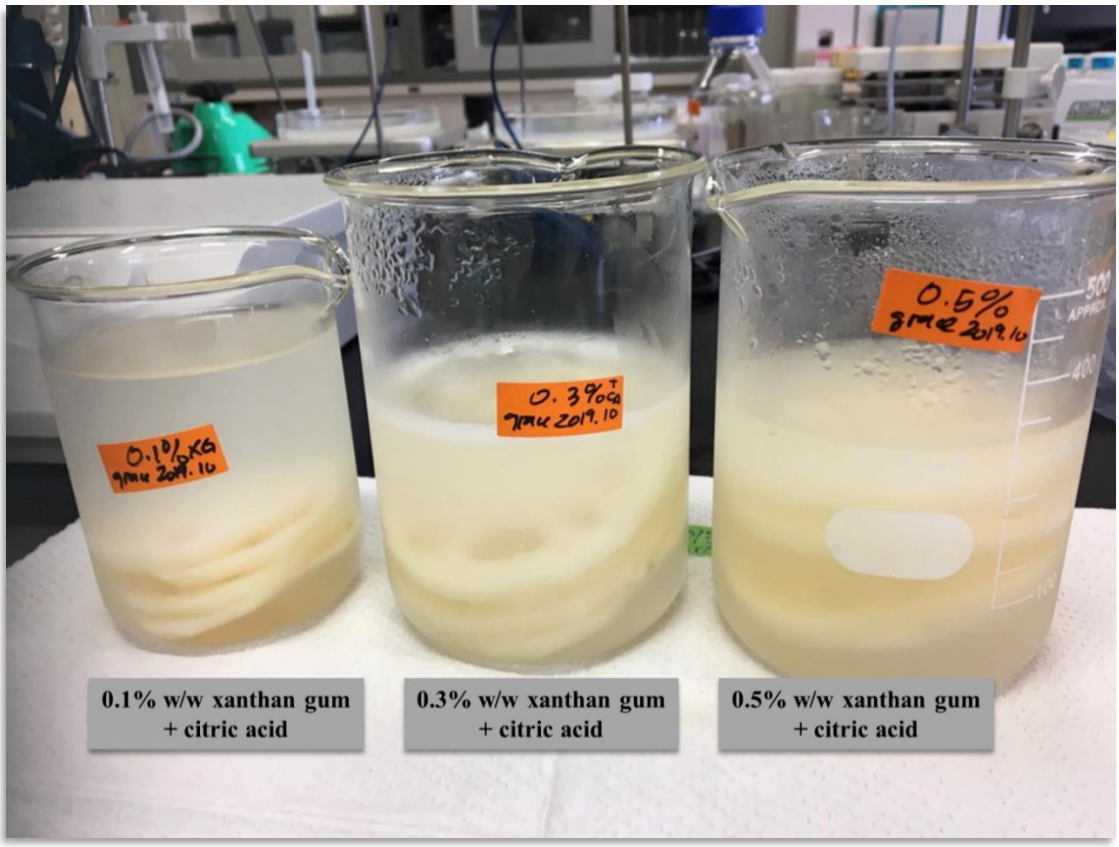


Fig. 5.1 Dipping method for the application of xanthan gum coatings to fresh-cut lotus root.

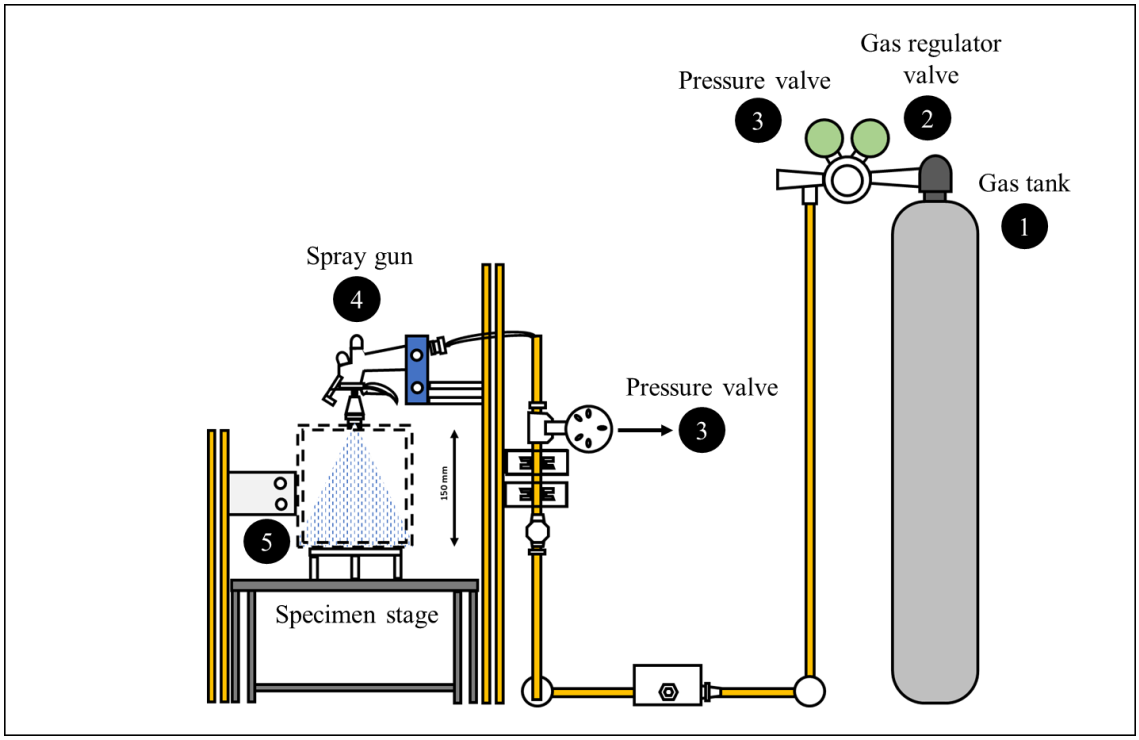


Fig. 5.2 Schematic diagram of the spray coating machine and its parts used for the spraying method.

5.3 Results and discussion

Changes in color of dipped and spray-coated lotus root samples during storage

The evaluation of color changes were performed due to the significance of visual quality in consumer's consideration during the purchase of fresh-products in the market. Figure 5.3 shows the calculated total color changes (ΔE) of fresh-cut lotus root samples applied with xanthan gum edible coatings through dipping and spraying. The results evidently proved that xanthan gum coatings both applied through dipping and spraying significantly reduced the total color changes (ΔE) of fresh-cut lotus root during 16 days of storage at 5°C. However, based on the obtained results, no significant difference were found between the two methods of edible coating application (dipping and spraying) in terms of reducing the total color changes during storage. The results achieved could be attributed to the small differences in the coating thickness for both dipping and spraying methods which ranged from 17 μm to 67 μm. Previously in Chapter 3, the significance of coatings thickness was emphasized as a critical parameter in the resulting barrier properties of edible coatings applied to fresh-cut lotus root.

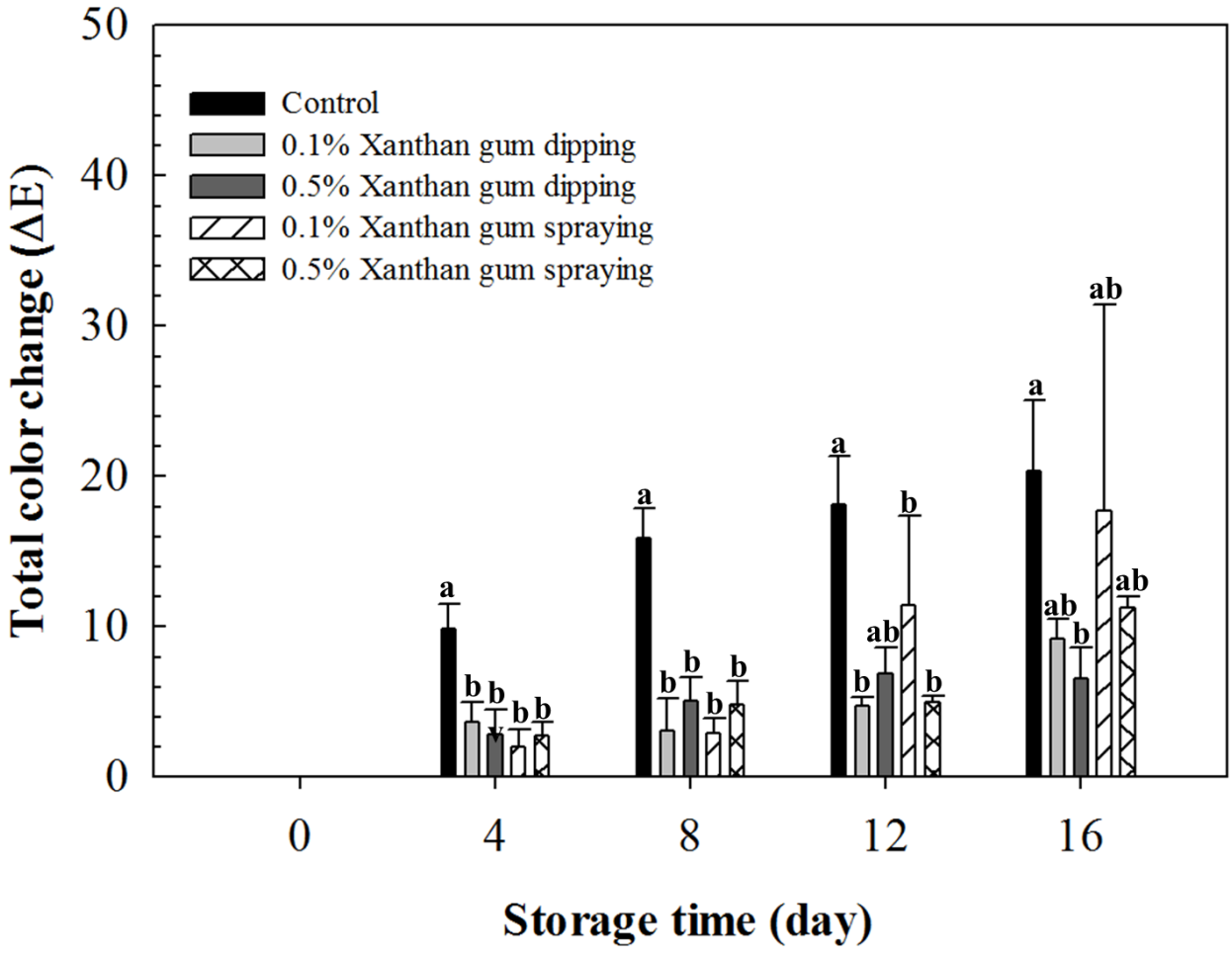


Fig. 5.3 Total color changes of fresh-cut lotus root during storage at 5 ℃. For a given storage period, different letters indicate significant differences between different treatments (Duncan's test; $p < 0.05$).

5.4 Conclusions

Dipping and spraying methods were evaluated as application techniques of xanthan gum coatings for fresh-cut lotus root in this Chapter. Results showed that both techniques were effective in significantly reducing total color changes (ΔE) during the storage of fresh-cut lotus root for 16 days at 5°C. However, no significant differences were observed between dipping and spraying methods, considering the reduction of color changes during storage.

Chapter 6

Cost analysis of hydrocolloid-based coating materials as edible coatings for fresh-cut lotus root

6.1 Introduction

Edible coatings can be sourced out from a wide variety of coating materials such as polysaccharides, proteins, and lipids.

Polysaccharides are good materials for edible coatings due to their excellent mechanical and structural properties; however, have weak poor ability against moisture transfer. Sources of polysaccharides include starch and derivatives, chitosan, pectin, cellulose and derivatives, alginate, and carrageenan (Lazi et al., 2015). Proteins are also another material for edible coating which have excellent gas and lipid barrier properties. Examples of these are casein, gelatin, collagen, and gluten. In addition, lipids are also sources of edible coatings which possess excellent gas and water barrier properties. These type of coatings can be sourced out from waxes, fatty acids, and shellac resins (Yai, 2008).

López et al. (2010) reported that the application of edible coatings has been acknowledged as an alternative or improved addition to the conventional packaging to improve food quality and contribute to food protection. Currently, several laboratory studies have been reported, and thus, to be able to scale up these processes, studies on feasibility and economical costs must be further considered.

In this chapter, the author aimed to understand the cost of the application of guar gum, xanthan gum, and chitosan coatings on its application to fresh-cut lotus root,

6.2 Materials and methods

6.2.1 Price of commercial polysaccharides used for calculations

The average commercial cost of xanthan gum, guar gum, and chitosan food-grade chemicals were surveyed online, and the market cost was considered for calculation.

6.2.2 Amount of coating material applied to fresh-cut lotus root

The amount of coating material that adhered to the fresh-cut lotus root was measured by holding the lotus root sample using clips and were dipped in the guar gum, xanthan gum, and chitosan solutions for 2 mins and then rapidly removed and drained over the glass beaker containing the xanthan gum solution. The glass beaker was placed on top of an analytical balance (Shimadzu Corporation UX 620H, Unibloc Toploading Balance, Kyoto, Japan) to measure the xanthan gum solution weight versus draining time. The amounts of the coatings solutions on the fresh-cut lotus root at 0, 20 s, 40 s, 60 s, 80 s, 100 s, 120 s, 140 s and 160 s draining time was the difference between the initial weight of the xanthan gum solution in the glass beaker and the weight recorded at the respective draining times. For this study, the amount of coating material after 120 s draining time was considered for further calculations.

6.3 Results and discussion

Cost and practicality are one of the major considerations in the application of coating materials on food products. In terms of economic costs, a coating material that contributes to minimal costs upon its application to a food material is highly desired.

Table 6.1 shows the calculated cost of the coating materials after the application on 1 kilogram of fresh-cut lotus root. Based on the calculated cost, an estimated 4.74 yen per 1 kilogram of lotus root will be incurred with the application of the guar gum coating material; 3.56 yen per 1 kilogram of lotus root for xanthan gum and 7.16 yen for 1 kilogram of lotus root for chitosan. The prices of guar gum, xanthan gum, and chitosan used for the calculations were based on the average commercial prices of these polysaccharides in the market. In this work, the calculated cost suggest a minimal additional cost with the application of guar gum, xanthan gum, and chitosan as coating materials for fresh-cut lotus root.

Despite the feasibility of edible coatings as barrier technologies for a wide variety of food products, Pashova et al. (2018) reported the consumer attitudes in Hungary towards the application of edible coatings in the food industry. The study demonstrated that approximately 80% of the Hungarian respondents were unfamiliar about edible coatings while only 10% of the respondents had the knowledge on edible coatings as eco-innovation strategies for food products. Based on the results, it is suggested that consumers remained to be uninformed regarding the use of edible coatings for food products. Therefore, it is strongly recommended that studies on edible coatings be strengthened, for the knowledge of consumers and the community.

Table 6.1 Cost analysis of coating materials applied to fresh-cut lotus root.

Coating material	**Commercial price, Yen (per g powder)**	**Amount of coating material needed, g**	**Estimated cost per 1 slice of lotus root*, Yen**	**Estimated cost per 1000 g of lotus root, Yen**
Guar gum	0.5	6.63	0.03	4.74
Xanthan gum	0.35	7.11	0.02	3.56
Chitosan	3.0	1.67	0.05	7.16

*1 slice of lotus root=7 g

6.4 Conclusion

In this chapter, the costs of xanthan gum, guar gum, and chitosan as coating materials for fresh-cut lotus root were evaluated. Results have shown that the application of the coating materials contributed to minimal cost to fresh-cut lotus root in the market. This suggest that these coating materials are feasible and practical for the use of edible coating for fresh-cut lotus root and other agricultural products in the future.

Chapter 7

Conclusion and prospective

Minimal processed fruits and vegetables such as fresh-cut products have been gaining popularity among consumers because of convenience and freshness. An example of a fresh-cut product gaining interest from researchers and industries is the fresh-cut lotus root (*Nelumbo nucifera*). Its white color, crisp texture, pleasant aroma, and high nutritional content make this rhizome appealing to consumers [1]. In Japan, the Ministry of Agriculture, Forestry, and Fisheries (2017) reported that the Ibaraki region produces the highest amount of lotus root yearly (39 million kg), which accounts for approximately 49% of the total production.

However, fresh-cut lotus root is prone to enzymatic browning and as a result, deteriorates its quality and shortens its shelf-life. Various strategies from researchers have been employed to improve the shelf-life of fresh-cut lotus root. Most of these strategies are mainly focused on chemical methods such as the use of antibrowning and preservatives and physical methods such as the application of gases and modified atmosphere packaging. But these methods have limited applications due to safety concerns of some chemicals, as well as the high-costs of equipment needed for modified atmosphere packaging (MAP). In addition, the author also reported that the use of acidic treatments led to the cell wall damage of fresh-cut lotus root, hence, resulted to tissue softening.

One of the alternative and promising approaches for the shelf life extension of fresh-cut lotus root is the application of edible coatings (Chapter 1). These coatings were reported to provide a semi-permeable barrier to gases and water vapor which reduced respiration rates, enzymatic browning, and water loss. Due to the limited information on the application of edible coating systems on fresh-cut lotus root, the author developed an

effective and low cost edible coatings from natural sources such as gums and marine polysaccharides and evaluated its effects on quality changes of fresh-cut lotus root such as color, texture, enzymatic activity, total phenols, and microstructural changes during 16 days of storage at 5°C.

In this work, it has been reported that edible coatings sourced out from natural polymers of guar gum, xanthan gum, and chitosan were proven to be effective in maintaining the storage stability of fresh-cut lotus root for 16 days storage at 5°C. Dipping and spraying methods of coating applications were also investigated in this work and storage stability parameters were also evaluated. However, based on the obtained results, no significant difference was found between the dipping and spraying method, in terms of the reduction of color changes during storage for 16 days at 5°C. Overall, the application of edible coatings is a promising strategy in extending the shelf life of fresh-cut lotus root. In the future, the author aims to widen the scope of the application of these coatings to other agricultural products which are prone to degradation during storage in the market.

List of Publications

[1] Lara, G. R., Uemura, K., Khalid, N., Kobayashi, I., Takahashi, C., Nakajima, M., Neves, M. A. 2019. Effects of acidic treatment on the physicochemical, microstructural and microbiological aspects of fresh-cut lotus root during storage. International Food Research Journal. 1505–1513, 26.

[2] Lara, G. R., Uemura, K., Khalid, N., Kobayashi, I., Takahashi, C., Nakajima, M., Neves, M. A. Layer-by-Layer Electrostatic Deposition of Edible Coatings for Enhancing the Storage Stability of Fresh-cut Lotus Root (*Nelumbo nucifera*). Food and Bioprocess Technology. Accepted.

[3]] Lara, G. R., Yakoubi, S., Villacorta, C., Uemura, K., Kobayashi, I., Takahashi, C., Nakajima, M., Neves, M. A. Spray technology applications of xanthan gum based edible coatings for fresh-cut lotus root (*Nelumbo nucifera*). Food Research International. In preparation.

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