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Effects of acidic treatment on the physicochemical, microstructural, and microbiological properties of fresh-cut lotus root during storage

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Abstract

Freshly cut slices of lotus root are a popular food item in various countries; however, problems such as enzymatic browning affect the storage stability of these products. In the present work, we evaluated for the first time the effect of low pH treatments on the overall quality of fresh-cut lotus root (physicochemical, microbiological, and morphological properties) during storage. The effect of acidic treatment, using citric acid solution at pH 2 or pH 4 at 2% w/w and 0.002% w/w, respectively, will help to improve our understanding of the mechanism of browning and the role of polyphenol oxidase in catalysing browning reactions. Treatments were prepared using citric acid and were applied to 5 mm thick fresh-cut lotus for 2 min. Samples were then stored for 16 days at 5°C. We found that the whiteness values (L^*) of pH 2 (68.76) and pH 4 (65.77) treated samples were significantly higher than the control (57.37), suggesting an enhancement of colour quality during storage. We also observed reduced polyphenol oxidase (PPO) enzyme activity for the treated samples as compared to the control (12.5, 16.5, and 17.13 U/min-mL, respectively), as well as reduced total phenolic content (46.5, 44.3, and 57.8 mg/100 g sample) and microbial counts. Despite these promising results, we also observed undesirable effects as a result of the pH treatments 2 and 4, including tissue softening and microstructural changes. However, we believe that our findings will be significant in the development of an optimised formulation for dipping treatments of fresh-cut lotus root products.

Introduction

An expansion of the market for fresh-cut fruits and vegetables has been reported in recent years (Qadri *et al*., 2015). Convenience and freshness are the two demands associated with freshly cut products (Francis *et al*., 2012). In addition, the International Fresh-cut Produce Association (Garrett, 1998) has emphasised that the recent increase in fresh-cut produce is related to the increased availability of health information and the typically busier lifestyles of consumers, thus putting a higher value on fresh, nutritious and convenient food products. However, fresh-cut products are prone to increased perishability and a shorter shelf life as compared to whole products due to the physical stress such as peeling, cutting, slicing, shredding, trimming and/or coring, and the removal of protective epidermal cells (Watada *et al*., 1996).

Elevated respiration rates and ethylene production, as well as oxidative browning reactions, lipid oxidation or enhanced water loss are some of the undesirable changes that occur in these products (Facundes *et al*., 2013). One of the most perceivable damages in fresh-cut fruits and vegetables is enzymatic browning (Roura *et al*., 2008). This phenomenon involves the oxidation of phenols into o-quinones, which are further induced by the action of

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polyphenol oxidase (PPO) (Altunkaya and Gokmen, 2008). The final products of oxidation are brown, red or black pigmentation (Garcia and Barrett, 2002). Factors involved in enzymatic browning include oxygen, PPO activity, and phenolic substrates (Shinoda, 2005; Holderbaum *et al*., 2010).

Recently, fresh-cut lotus (*Nelumbo nucifera*) root slices have drawn the attention of researchers and the industry as a novel minimally-processed vegetable (Zhang *et al*., 2013). However, fresh-cut lotus root is prone to enzymatic browning, thus deteriorating its quality and shortening its shelf-life. Xing *et al*. (2010) found that browning and oxidation occur in untreated fresh-cut lotus root slices after two days of storage, making this product undesirable in the market, while Hu *et al*. (2014) reported that PPO and phenylamine ammonia lyase (PAL) activity increased after one day of storage in fresh-cut lotus root products, which resulted in increased browning indices. However, to the best of our knowledge, there are few available studies on the control of fresh-cut lotus root enzymatic browning. Xing *et al*. (2012) studied the combined effects of anti-browning agents, cinnamon oil fumigation, and moderate vacuum packaging. Zhang *et al*. (2013) considered carbon monoxide on browning of fresh-cut lotus root. Son *et al*. (2015) evaluated the effects of ascorbic acid, heat treatment, and modified-atmosphere packaging. However, no studies have reported the effects of low pH treatments on the overall quality of fresh-cut lotus root during storage. Chiabrando and Giacalone (2012) found the use of 1% ascorbic acid, 1% citric acid and 1% calcium chloride was effective in reducing enzymatic browning in fresh-cut apples. Similar results were also reported by Sanchís *et al*. (2017), who emphasised the combined use of 1% ascorbic acid, 1% citric acid, and controlled atmosphere packaging for decreasing flesh colour browning and loss of firmness in fresh-cut persimmons during storage. The present work thus investigated the effect of acidic treatments on the physicochemical, microstructural, and microbiological properties of fresh-cut lotus root during 16 days of storage.

Materials and methods

Chemicals

The chemical reagents anhydrous citric acid, polyvinylpyrrolidone, catechol, sodium phosphate, Folin-Ciocalteu, gallic acid, and standard method agar were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Anhydrous citric acid was used to adjust the pH for the acidic treatments.

Preparation of acidic solutions

For the preparation of the pH 2 and pH 4 treatments, 2 g and 0.002 g of anhydrous citric acid, respectively, were weighed and added to Milli-Q water to obtain a final weight (w/w basis) of 100 g. The citric acid solutions were magnetically stirred (hot stirrer, HS 360 H; As One, Tokyo, Japan) for 1 min at room temperature (25°C) to assure the complete dissolution of the citric acid. The resulting pH of each citric acid solution was verified (827 pH lab; Metrohm, Herisau, Switzerland) before use as the acidic treatment for the fresh-cut lotus root.

Sample preparation

Three experimental treatments (control, pH 2, and pH 4) were prepared. The control consisted of freshly sliced lotus root without any pH adjustment. The lotus root samples were obtained from a local supermarket in Tsukuba, Japan, and were confirmed to be fresh and of good quality by evaluating their whiteness and the length of duration on the supermarket shelf. The samples were thoroughly washed under tap water, then peeled and sliced into 5-mm thick sections. The dipping technique was used for the acidic treatment of the fresh-cut lotus root. Five slices (5-mm thick) of fresh-cut lotus root per treatment were immersed for 2 min. Preliminary studies were performed prior to this experiment with regards to dipping time; only slight differences in the storage of fresh-cut lotus root were found with a longer dipping time. The fresh-cut lotus root samples were then quickly dried by allowing the excess citric acid to drip from the surface of the samples. The fresh-cut lotus root slices were packed into polyethylene bags (60 μm × 160 mm × 260 mm; GT-1626, Sigma Tube, Tokyo, Japan) and were stored for 16 days at 5°C to evaluate the storage stability parameters of colour, texture, pH, enzymatic activity, total phenolic content, and microbiological properties.

Evaluation of physicochemical changes during storage

Colour

Colour changes during the 16 days of storage were monitored using a spectrophotometer (CM-5; Konica Minolta, Tokyo, Japan). The 16-day storage time was found to be sufficient to observe the colour, textural, enzymatic, and microstructural changes and to determine the stage at which enzymatic browning occurs after the acidic treatments. The L^* values were used to express lightness or darkness of the treated and untreated samples.

Texture

The textural changes were evaluated using the Texture Profile Unit (TPU 2DL; Yamaden, Tokyo, Japan). The 5-mm thick samples were longitudinally compressed 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).

pH measurement

The pH measurement was adapted from Son *et al*. (2015) with a few modifications. Twenty grams of each fresh-cut lotus root sample was homogenised in 40 mL Milli-Q water for 30 s using a multipurpose blender (Vitaprep 3; Vitamix Corp., USA). The pH of the blended samples was determined using a digital pH meter (827 pH lab; Metrohm, Herisau, Switzerland).

Weight loss

The weight loss of the samples during storage was also evaluated. The values were expressed as percentage losses from the initial weight of the sample using Equation 1, as follows:

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

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

Polyphenol oxidase activity

The method for the determination of the enzymatic activity of PPO was based on the standard procedures reported by Son *et al*. (2015) with a few modifications. Ten grams of each fresh-cut lotus root sample per treatment was homogenised in sodium phosphate buffer (0.2 mol/L, pH 7.0 with 2% polyvinylpyrrolidone) at a ratio of 2.0 mL of buffer per 1 g of lotus root in an ice bath. The homogenates were centrifuged using a high-speed refrigerated centrifuge (Tomy MX -307; Tomy Seiko, Tokyo, Japan) at 12,000 g for 10 min at 4°C. The supernatant was separated from the filtrate and was reserved for analysis. Enzymatic analysis was performed by evaluating 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 then added to 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 alone (catechol in sodium phosphate buffer) was used as a reference. The enzymatic activity (units/(min-mL) enzyme) was determined using the linear section of the activity curve. A change in 0.001 in the absorbance value per min was defined as 1 unit of PPO enzyme activity.

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 was added to 25 mL Milli-Q water after crushing with a mortar and pestle. Centrifugation was performed for 15 min at 5,000 *g*. One millilitre of the supernatant was mixed with 5 mL Folin-Ciocalteu solution (1 mL of FC reagent in 10 mL Milli Q water). Between 0 and 8 min of reaction time, 4 mL (7.5% w/v sodium carbonate solution) was slowly added. The samples were incubated for 2 h at 30°C in a controlled incubator (Panasonic cool incubator; ICI-200, Tokyo, Japan). The spectrophotometer was used to measure the absorbance of the solution at 760 nm. Gallic acid was used as a standard for the analysis. Acid solutions at concentrations ranging from 0.01 - 0.1 mg/mL dissolved in Milli-Q water were prepared prior to plotting the standard curve. The results were expressed as mL of gallic acid per 100 grams of fresh-cut lotus root.

Evaluation of microbiological count during storage

Prior to the microbiological analysis, a slice of each of the fresh-cut lotus root samples (control, pH 2 treatment, and pH 4 treatment) was cut into three portions to ensure the same flora of microorganisms were present. For the analyses, one slice of the lotus root (10 g) was aseptically removed from the polyethylene bags and transferred into a sterile stomacher bag. Ten millilitres of Milli-Q water were added to the sample prior to homogenisation. These samples were then homogenised in the stomacher for 1 min. One millilitre of the sample was pipetted for serial dilutions. One millilitre per dilution (10^{-1} to 10^{-6}) was placed onto petri dishes, onto which plate count agar (PCA) was poured before incubating for 24-48 h at 37°C. The resulting microorganism colonies were manually counted. The results were expressed as log CFU/g (colony forming units per g of lotus root sample). All microbiological tests were performed at least in duplicate on a clean bench at room temperature.

Evaluation of morphological changes during storage

Morphological analyses were performed using a scanning electron microscope (SEM Miniscope 1000; Hitachi, Tokyo, Japan). Prior to microscopy, 2 g of

samples per treatment were sliced into 1 mm^3 cubes and oven-dried at 100°C for 5 h to achieve 16% ± 2.0 total solids content (w/w). After drying, the samples were mounted onto an aluminium sample probe for magnification at 500×. The samples were observed after 16 days storage at 5°C.

Statistical analysis

All experiments were performed at least in triplicate, and the results were reported as the mean ± standard deviation (SD) of the measurements. SPSS Statistics (IBM Statistics 22; New York, USA) was used to perform one-way analysis of variance (ANOVA). The Duncan multiple range test was used as a post hoc test at a significance of 95% probability. The significant difference among the treatments ($p < 0.05$) was indicated using different superscript letters.

Results and discussion

Changes in colour of acid-treated lotus root samples during storage

Colour is one of the most important quality attributes that consumers take into consideration when purchasing a food product. Table 1(a) shows the effect of pH 2 and pH 4 on the whiteness (L^* values) of the lotus root samples. L^* values were used to indicate the lightness of the samples. A higher whiteness (L^* value) indicates a brighter sample surface (Du *et al*., 2009). A decreasing whiteness (L^* values) was observed in all samples, which can be attributed to enzymatic browning. After four days of storage, the control samples showed significant differences in terms of colour as compared to the samples treated with pH 2 and pH 4. This suggests that the control samples exhibited the highest rate of enzymatic browning as compared to the pH 2- and pH 4-treated samples. However, no significant difference ($p > 0.05$) in whiteness (L^* values) was observed after 16 days of storage in any of the samples, indicating that browning occurred in all the samples.

Altunkaya and Gokmen (2008) found that citric acid functions via two inhibitory mechanisms: (1) by lowering the pH below that required for optimal PPO activity at pH 4.0-8.0 and (2) by chelating copper. In the present work, citric acid was used to lower the pH to determine whether two pH values influenced the storage quality of fresh-cut lotus root. We found that a lower pH helped to reduce PPO activity, thus reducing enzymatic browning, observed in the pH 2 and pH 4 samples. However, after 12 days of storage, enzymatic browning also occurred in the pH 2 and pH 4 samples, which can be attributed to the partial inactivation of PPO during acidic treatments. Li *et al*. (2018) reported that at pH 2, the PPO activity in fresh-cut potato only decreased to 46.10%, suggesting only the partial inactivation of the enzyme. Ali *et al*. (2015) confirmed the same results in their study, where 1% citric acid was found to reduce the browning indices of lettuce heads during eight days of storage in comparison to the untreated samples. However, despite the reduction of browning indices in the citric acid treated samples, browning was observed after only two days of storage. Ali *et al*. (2015) proposed that citric acid does not completely avoid browning, but delays the development of browning, indicating its action as a PPO inhibitor. Based on our results, pH 2 treatment showed superior results than the pH 4 treatment in terms of enhancing the colour quality of the samples.

Changes in texture of acid-treated lotus root samples during storage

Toivonen and Brummell (2008) demonstrated that the disruption of sub-cellular compartmentalisation occurs at the surfaces of the sliced lotus roots, which results in undesirable reactions. In addition, changes in turgor pressure and the structure and composition of cell walls, particularly cell wall polysaccharides, results in tissue softening (Pinheiro and Almeida, 2008; Krall and McFeeters, 1998). Table 1(b) shows the effect of pH on the texture, indicated as hardness, of the fresh-cut lotus root samples during 16 days of storage.

A decreasing hardness in all samples (Table 1(b)) indicates tissue softening. Based on these results, the pH 2-treated samples showed the lowest hardness values after 16 days of storage, followed by the pH 4-treated samples and the control. Son *et al*. (2015) found similar decreasing hardness values during storage, which can be attributed to weight loss associated with loss of water (Bico *et al*., 2009).

Changes in pH of acid-treated lotus root samples during storage

Changes in the pH values of the treated and the control fresh-cut lotus roots were also evaluated in the present work, as shown in Table 1(c). Our results showed an increase in the pH of the untreated, pH 2-treated, and pH 4-treated samples during the 16 days of refrigerated storage; however, significant differences ($p < 0.05$) were observed for the pH 2- and pH 4-treated samples. Gil *et al*. (2006) and Facundes *et al*. (2013) compared the changes in quality and nutrient retention in fresh-cut apples during storage. Their results showed an increase in pH for fresh-cut apple slices during storage, which was attributed to a

Table 1. Physicochemical changes of fresh-cut lotus root treated at different pH's during storage at 5°C.

Treatment	Storage time (day)				
	0	4	8	12	16
a) Colour (L*)					
Control	68.26 ± 0.16^{a}	64.84 ± 0.67^{b}	57.37 ± 1.53^{c}	53.04 ± 0.84^{b}	52.72 ± 1.45^{a}
pH 2.0	69.96 ± 0.95^{a}	69.26 ± 1.23^{a}	68.76 ± 1.09^{a}	58.87 ± 1.09^{a}	54.7 ± 2.22^{a}
pH 4.0	70.14 ± 0.73^{a}	69.44 ± 0.16^{a}	65.77 ± 1.40^{b}	60.26 ± 2.56^{a}	54.5 ± 1.24^{a}
b) Texture (N)					
Control	44.19 ± 2.33^{a}	35.7 ± 3.34^{b}	21.66 ± 1.37^{a}	17.52 ± 1.92^{a}	16.08 ± 1.31^{a}
pH 2.0	46.01 ± 3.15^{a}	37.01 ± 1.48^{a}	21.13 ± 2.42^{a}	18.55 ± 2.87^{a}	12.24 ± 0.66^{c}
pH 4.0	44.14 ± 0.40^{a}	30.65 ± 0.87^{c}	24.34 ± 0.77^{a}	18.82 ± 2.39^{a}	13.48 ± 0.66^{b}
c) pH					
Control	6.42 ± 0.07^{a}	6.45 ± 0.12^{a}	6.48 ± 0.01^{a}	6.49 ± 0.03^{b}	6.57 ± 0.17^{a}
pH 2.0	4.81 ± 0.03^{b}	4.9 ± 0.11^{b}	5.12 ± 0.08^{b}	5.15 ± 0.06^{c}	5.43 ± 0.00^{b}
pH 4.0	6.33 ± 0.02^{a}	6.40 ± 0.03^{a}	6.55 ± 0.03^{a}	6.74 ± 0.03^{a}	6.76 ± 0.23^{a}
d) Weight Loss (%)					
Control	0.00 ± 0.00	0.92 ± 0.43^{a}	2.57 ± 0.35^{a}	2.93 ± 0.86^{a}	4.81 ± 0.71^{a}
pH 2.0	0.00 ± 0.00	2.03 ± 0.23^{a}	3.55 ± 0.67^{ab}	5.33 ± 0.98^{ab}	6.75 ± 1.75^{a}
pH 4.0	0.00 ± 0.00	0.66 ± 0.76^{a}	1.28 ± 0.91^{b}	1.99 ± 0.37^{b}	4.09 ± 2.19^{a}
e) PPO Enzymatic Activity (U/min-mL)					
Control	19.25 ± 1.06^{a}	18.5 ± 0.35^{a}	17.25 ± 1.06^{a}	17.13 ± 0.71^{a}	17.13 ± 0.35^{a}
pH 2.0	17.25 ± 3.89^{a}	13.5 ± 1.41^{b}	13.25 ± 1.06^{b}	12.5 ± 1.41^{b}	12.5 ± 0.00^{b}
pH 4.0	16.25 ± 1.77^{a}	18.75 ± 0.35^{a}	18.25 ± 0.35^{a}	16.5 ± 0.71^{a}	16.5 ± 0.71^{a}
f) Total Phenolic Content (mg/100 g fresh weight)					
Control	57.8 ± 0.64^{a}	71.78 ± 1.24^{a}	73.53 ± 0.54^{a}	76.77 ± 1.68^{a}	71.45 ± 0.21^{a}
pH 2.0	46.5 ± 2.83^{b}	69.5 ± 0.75^{a}	72.0 ± 0.63^{b}	72.9 ± 0.71^{a}	68.3 ± 2.06^{a}
pH 4.0	44.3 ± 1.41^{b}	69.4 ± 2.39^{a}	74.2 ± 0.76^{a}	75.6 ± 1.51^{a}	71.3 ± 0.21^{a}

Different superscript letters indicate significant differences with 95% probability.

deterioration of characteristics such as colour, texture and nutritional components.

Generally, lotus root cultivars consist of 13% protein, 2.3% crude fat, 66% carbohydrates and 2.8% fibre (Park *et al*., 2009). A possible breakdown of these components could have caused the increase in pH of the samples during storage. Initially, the pH of the samples treated with pH 2 increased to 4.81, then increased further with storage time, while the pH of samples treated with pH 4 had an initial pH of 6.33. This could also be a possible explanation for enzymatic browning during storage, in which the monitored pH increased towards the optimal pH range for PPO that is pH 4.0 - 8.0 (Yoruk and Marshall, 2003).

Changes in weight loss of acid-treated lotus root samples during storage

The removal of the peel during post-harvest processing results in agricultural commodities that are more susceptible to being perishable (Garcia and Barrett, 2002). The weight loss of freshly cut lotus root samples (control, pH 2, and pH 4) during 16 days of storage is presented in Table 1(d). As observed, the weight loss of the control, pH 2-treated, and pH 4-treated samples increased during storage. Son *et al*. (2015) reported similar results of increased weight loss during storage, which could be attributed to water loss or dehydration. However, we observed a higher weight loss in the samples treated with pH 2 in comparison with the control and pH 4 samples. We hypothesised that at a low pH, such as pH 2, cell wall disruption and cells shrinkage can occur, thereby causing water loss and further affecting the product's weight loss.

Changes in enzymatic activity of polyphenol oxidase (PPO) in acid-treated lotus root samples during storage

Wang *et al*. (2009) found that the optimum pH and temperature of PPO activity of lotus roots were 7.5 and 35°C, respectively. However, the effects of pH on storage stability parameters such as colour, texture, microbiological, and morphological properties have not been reported. Table 1(e) shows the variations in PPO enzymatic activity between the

untreated, pH 2-treated, and pH 4-treated samples. As observed in Table 1(e), a smoother decreasing trend of enzymatic activity was visibly observed in the control and pH 2 samples throughout the 16 days of storage, while more marked increases and decreases were observed for the pH 4 sample. The same PPO enzymatic activity trend was reported by Xing *et al*. (2010) in fresh-cut lotus root, which can be attributed to the stability of the PPO enzyme being influenced by treatments, such anti-browning agents, during storage.

The higher PPO activity at day 0 could be attributed to the wounding and slicing processes (Xing *et al*., 2012). However, the lower PPO activity of pH 2 and pH 4 as compared to the control could be due to the lowering of the pH resulting from the acid treatments. The experimental results showed that the pH 2-treated samples had the lowest enzymatic activity, as compared to other treatments, at days 0, 4, 8, 12 or 16 of storage. The lower enzymatic activity could be related to the lower degree of browning, previously reported as L^* values. Our results also showed that the enzymatic activity of the untreated samples as compared to pH 4-treated samples show almost no difference at storage times of 0, 4, 8, 12, and 16 days, which suggest that pH 4 treatment was less effective in suppressing PPO activity.

Changes in the total phenolic content of acid-treated lotus root samples during storage

Slicing is one of the abiotic stress processes that occur during fresh-cut lotus preparation (Hu *et al*., 2014), which could lead to the accumulation of phenolic compounds (Dixon and Paiva, 1995; Chalker-Scott, 1999; Reyes and Cisneros-Zevallos, 2003). Table 1(f) shows the increasing phenolic content of untreated and treated fresh-cut lotus root samples up until 12 days of storage and a decrease after 16 days of storage. The increase in the phenolic contents of the samples can be attributed to the wounding of the tissues during the cutting process (Hu *et al*., 2014). Our results show that, immediately after acidic treatment, the total phenolic contents of the pH 2- and pH 4-treated samples were lower than that of the control. The pH 2-treated samples exhibited a lower total phenolic content than the untreated and pH 4-treated samples. This suggests that the pH 2 and pH 4 treatments reduced the production of phenolics resulting from slicing and wounding during post-harvest processing, thus decreasing the amount of substrate available for oxidation. However, after 12 days of storage, we found that there was no significant difference between the control and the pH 2- and pH 4-treated samples, which suggested that phenolics, which cause enzymatic browning, had been produced; thus resulting in colour changes in all the treatments during storage.

Changes in microbiological count of acid-treated lotus root samples during storage

The presence of water, starch, and vitamins in lotus root make it an excellent source of nutrients for the proliferation of microorganisms during post-harvest processing and storage (Du *et al*., 2009). Thus, the microbiological quality of the samples must be assessed for the effective preservation of fresh-cut produce (Bico *et al*., 2009).The total microbial counts for the untreated, pH 2-treated, and pH 4-treated samples are presented in Figure 1. Our results show that, on day 0, the untreated (control) and pH 4-treated samples had higher microbial counts (6 log CFU/g) than the pH 2-treated sample

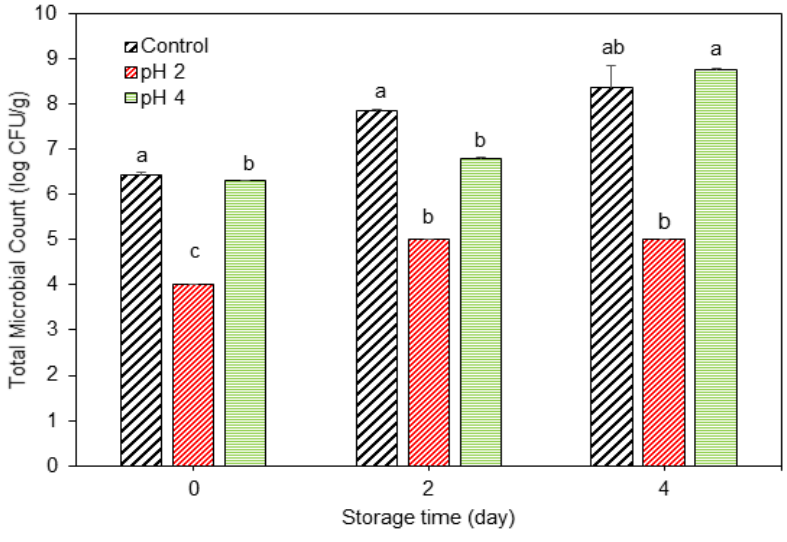


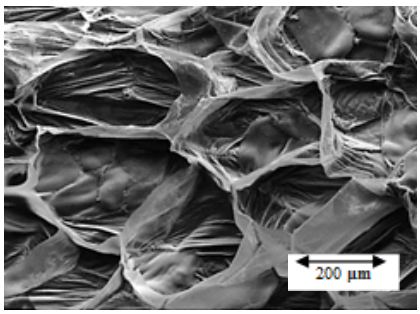
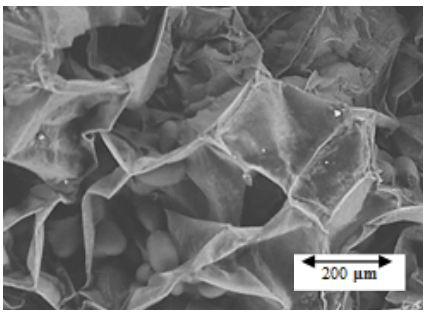
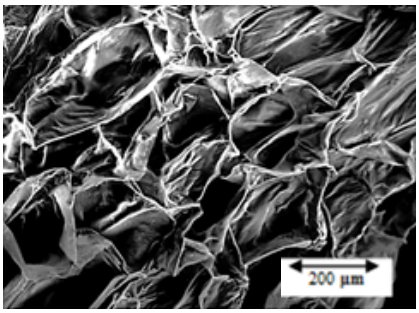
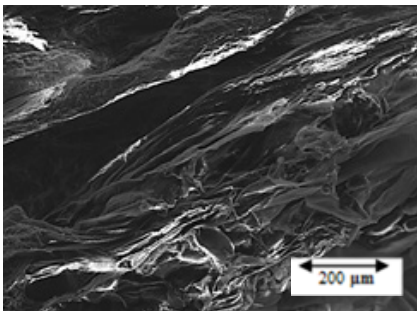
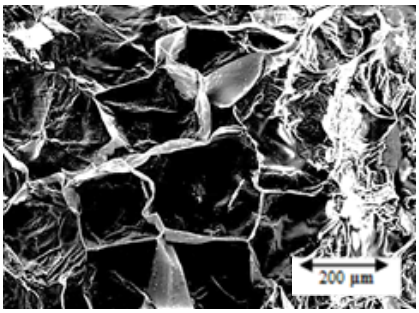
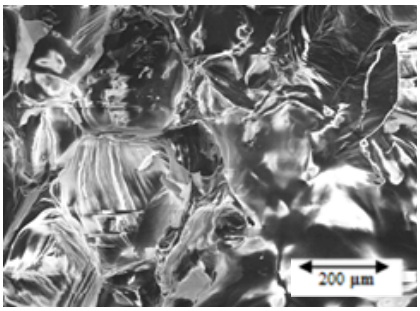
Figure 1. Total microbial counts of fresh-cut lotus root treated at different pH's, stored at 5°C. Different letters indicate significant difference at 95% probability.

(4 log CFU/g). These results indicate that pH 2 treatment decreased the initial microbial count from the control by 2 log reductions. An increase of the microbial counts was observed for both the control (7 log CFU/g to 8 log CFU/g) and pH 4-treated (6 log CFU/g to 8 log CFU/g) samples from day 2 to 4, with no observable increase in the pH 2 samples (5 log CFU/g). The storage experiment was terminated after day 4 due to excessive microbial growth in the control and pH 4-treated samples. These results suggest that pH 2 treatment could reduce the microbial counts in fresh-cut lotus root.

Morphological analyses using scanning electron microscopy

Despite the importance of the microstructure and its degradation, its role in the maintenance of food quality has been largely underestimated (Tu *et al*., 2015). To evaluate the effect of low pH treatments (pH 2 and pH 4) on the microstructure of the fresh-cut lotus root samples, morphological analyses were performed. SEM micrographs, presented in Table 2, show the results of the microstructure analysis of the control, pH 2-treated, and pH 4-treated samples between days 0 and 16 of storage. From the micrographs, it can be observed that the cell walls of the control samples remained intact and were visibly defined during the 16 days of storage. Meanwhile, the pH 2-treated samples showed cell wall breakdown with increasing storage time. Initially, the pH 2-treated samples exhibited distortion and less defined cell walls and these were continuously observed until day 16, which most likely indicated cell wall damage caused by low pH conditions. The cell walls of pH 4-treated samples showed similar distortions to those observed in the pH 2-treated samples, which also suggests partial cell wall damage. Tu *et al*. (2015) found that raw lotus root samples were composed of defined and organised individual cells, which is correlated with the results obtained in the present work. However, after freezing, the cells appeared torn and were irregularly shaped with a partial loss of amorphous materials and tissue distortions (Tu *et al*., 2015). Our findings suggest that the acidic

Table 2. SEM micrographs of fresh-cut lotus root treated at different pH's, at day 0 and after storage for 16 days at 5°C.

Treatment	Storage time (day)	
	0	16
Control		
pH 2		
pH 4		

treatments had a similar effect on cell wall damage and disruption. Krall and McFeeters (1998) reported that pectin, a major component of the cell wall, rapidly degraded at pH 2.5 - 4.5. Fraeye *et al*. (2007) also reported that the acid hydrolysis of pectin occurs at pH values as low as 3.8. As such, we speculated that, at pH 2, cell wall damage would occur, as can be observed in Table 2. We propose that this cell wall damage resulted in aggressive tissue softening in the pH 2-treated samples.

Conclusion

One of the major problems that fresh-cut lotus root slices encounter is enzymatic browning. As far as the authors were aware, there are currently no other studies focusing on the specific effect of low pH treatments on the overall quality of fresh-cut lotus root, in particular the impact on the microstructural properties of this food product. Based on our results, we found out that lowering the pH of the immersion treatments to pH 2 was the most effective method for controlling enzymatic browning in fresh-cut lotus root. However, this method may also result in tissue softening and microstructural changes during storage. Our study elucidated the effect of low pH on the storage stability of fresh-cut lotus root. These findings provide a basis for the formulation of dipping treatments for fresh-cut lotus root, as well as other agricultural products.

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