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Effect of chitosan molecular weight on the functional properties of chitosan-maltose Maillard reaction products and their application to fresh-cut *Typha latifolia* L.

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ABSTRACT

The objective was to evaluate antimicrobial, antioxidant and copper-chelating activities of Maillard reaction products (MRP) prepared from maltose and different molecular weight chitosan, and their effects on preservation of fresh-cut *Typha latifolia* L. (TLL). LMRP (maltose and low molecular weight chitosan MRP) showed the highest browning and UV absorbance as well as fluorescence intensity. The DPPH radical scavenging activity, reducing power and copper-chelating activity of chitosan-maltose MRP varied depending on the chitosan molecular weight. HMRP (maltose-high molecular weight chitosan MRP) exhibited better effects on inhibiting PPO activity and discoloration, alleviating declines of total soluble solids and ascorbic acid content of fresh-cut TLL. LMRP and MMRP (maltose-medium molecular weight chitosan MRP) effectively decreased weight loss and maintained firmness of TLL, respectively. These results indicated that molecular weight of chitosan had a great impact on the functional properties of chitosan-maltose MRP and their application to be used as a preservative.

1. Introduction

In recent years, fresh-cut common cattail (*Typha latifolia* L. (TLL)) has been attracting increasing attention of research and industry as a novel minimally processed vegetable. However, TLL is a highly perishable product and tends to lose quality immediately after harvest. Its shelf life is short because of its tendency to turn brown and having no protection to avoid weight loss or microbial contamination. Therefore the short shelf-life of TLL is a block to the distribution and marketing of the fresh product.

Chitosan is the cationic (1-4)-2-amino-2-deoxy-<beta>-D-glucan; in general its degree of acetylation is close to 0.20, when it is isolated from marine chitin. It is fully safe when ingested or brought into contact with human tissues (Busilacchi, Gigante, Mattioli-Belmonte, Manzotti, & Muzzarelli, 2013; Muzzarelli, 1996, 2009, 2010; Muzzarelli et al., 2012). Due to its antioxidative (Ai, Wang, Xia, Chen, & Lei, 2012), antimicrobial activities (Vallapa et al., 2011), film-forming properties (Bonilla, Atarés, Vargas, & Chiralt, 2013), chitosan has received attention as a potential food preservative of natural origin in fruits and vegetables (Badawy & Rabea, 2009; Lin et al., 2011; Xing et al., 2010). The characteristics of chitosan depended on the degree of deacetylation, the distribution of acetyl groups, chain length, and molecular weight (MW) distribution (Terbojevich, Cosani, Focher, Naggi, & Torri, 1992). Further, it has been reported that biological activities of chitosan including antimicrobial and antioxidant activities are dependent on MW (Kim & Thomas, 2007; Mellegard, Strand, Christensen, Granum, & Hardy, 2011).

Chemical modifications have been used to improve the properties of chitosan. To overcome the limited solubility, Yang, Chou, and Li (2002) prepared water-soluble chitosan derivatives through the reductive N-alkylation of chitosan with different disaccharides (lactose, maltose or cellobiose). These disaccharide chitosan derivatives were demonstrated to exhibit antibacterial and antioxidant activity in a system with a pH at 7.0 or higher (Lin & Chou, 2004). Through regioselective glycosylation with the disaccharide, β-maltoside branches were introduced to synthesize non-natural branched chitosan. The disaccharide branch significantly influenced the properties in many respects; for instance, it increased affinity for water more than monosaccharides (Kurita, Akao, Yang, & Shimojoh, 2003). Owing to the formation of potential detrimental products, chemical modifications are generally not preferred for food applications (Kanatt, Chander, & Sharma, 2008). The Maillard

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reaction, resulting from the initial condensation between amino group of chitosan and carbonyl moiety of reducing sugars, aldehydes or ketones, is one of the main reactions taking place in food. Maillard reaction generally produces a far-ranging of products with antioxidative (Sun, Zhao, Cui, Zhao, & Yang, 2010) and antimicrobial properties (Rufián-Henares & Morales, 2006). Maillard reaction products (MRP) of amino acid-sugar have been found to exhibit strong antioxidant activity. The MRP, which produced from reactions of glucose-arginine, valine, or histidine (Miranda, Rakovski, & Were, 2012), ribose-glycine (Jalbout, Shipar, & Navarro, 2007), glutathione-glucose or fructose (Billaud, Brun-Merimee, Louarme, & Nicolas, 2004), casein-glucose (Gu et al., 2010), ribose-lysine and fructose-lysine (Vhangani & Wyk, 2013) model systems, have been associated with the formation of compounds with strong antioxidant activity.

Having amino groups makes chitosan a candidate to react with the carbonyl group of reducing sugars, and allows it to participate in the Maillard reaction. Chitosan-sugar conjugates have been regarded as a kind of functional biopolymers having outstanding antimicrobial effects and antioxidant properties. Chitosan-glucosamine MRP exhibited higher metal-ion chelating capacity and antibacterial activities compared with acid-soluble chitosan (Chung, Kuo, & Chen, 2005). Chitosan-glucose MRP formed by Maillard reaction have been reported to exhibit antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens* and *Bacillus cereus* (Jiang, Feng, & Li, 2012; Kanatt et al., 2008; Rao, Chawla, Chander, & Sharma, 2011). Meanwhile, chitosan-glucose MRP showed excellent antioxidative properties (Chang, Chen, & Tan, 2011; Gao, Zhu, & Zhang, 2013). In addition, chitosan-xylose MRP exhibited antioxidant activities, copper-chelating property and good inhibitory effect on polyphenol oxidase (Huang, Huang, Huang, & Chen, 2007; Zhu et al., 2013). Studies showed that, MRP between chitosan and polysaccharides were promising antioxidative and antibacterial preservatives for food storage (Li, Shi, Jin, Ding, & Du, 2013).

Although many studies of chitosan-sugar MRP have been performed, studies of the effects of chitosan molecular weights on the properties of MRP are limited. Besides the research on the application of MRP to vegetables is also few. The present work was designed to evaluate if chitosan molecular weights affected functional properties of chitosan-maltose MRP and their application to fresh-cut TLL.

2. Materials and methods

2.1. Chemicals

Three kinds of food grade chitosans with different molecular weights (DMWC) (1020 ± 74 kDa (HMWC), 495 ± 36 kDa (MMWC) and 190 ± 32 kDa (LMWC) with deacetylation degrees of 81 ± 3%) used in this study were purchased from Golden-Shell Pharmaceutical Co., Ltd. (Zhejiang, China). d-(+)-Maltose (analytical grade) was purchased from Tianjin Chemical Reagent Co., Ltd. (Tianjin, China). 2, 2,-Diphenyl-1-picryl hydrazyl (DPPH) and potassium fericyanide were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade, unless otherwise stated.

2.2. Preparation of MRP and TLL samples

The chitosan-maltose Maillard reaction products were prepared by the reaction of chitosan with maltose. According to the methods of Kanatt et al. (2008) with some modifications, one gram of chitosan was dissolved in 100 ml of acetic acid (1%) in which 1.0% of maltose was added. The pH value of each solution was adjusted to 6.0 by adding 1 M sodium hydroxide. The mixtures were then heated in a water bath at 100 °C for up to 6 h. Samples were withdrawn at given time intervals, and immediately cooled in iced water. MRP, which were prepared by high, medium and low molecular weight chitosan with maltose, was designated as HMRP, MMRP and LMRP, respectively. The MRP-*x* was expressed for Maillard reaction products prepared by heating chitosan with maltose for *x* h.

Fresh TLL was purchased from the local wholesale market at Huai'an, PR China. They were selected for uniformity of size and ground color, freedom from defects and mechanical damage. After washed with running water and peeled manually, they were cut into about 5 cm length segments using a sharp knife. Four different treatments were used: (1) control; (2) 1% maltose coating; (3) 1% DMWC coating, and (4) 1% MRP coating. TLL samples were dipped into the solution for 5 min. Samples dipped in distilled water were used as control. Treated samples were kept over a plastic sieve for 30 min and treated by a fan generating low-speed air to hasten drying. The excess solution on the surface was absorbed by a tissue paper. The treated samples of 100 g were placed and sealed in 18 cm × 20 cm bags of low density polyethylene (30 μm thickness). Then the samples were stored at 20 °C for 15 days. During the storage, color, polyphenol oxidase (PPO) activity, weight loss, firmness, total soluble solids (TSS), ascorbic acid content (AAC) and microbial group were measured at two-day intervals.

2.3. Spectrophotometric analysis of MRP

Browning and UV absorbance of the MRP were measured according to the method of Rao et al. (2011). Appropriate dilution was made using distilled water. And then UV absorbance and browning intensity were measured at 280 and 420 nm respectively. The fluorescence intensity was measured at an excitation wavelength of 346 nm and emission wavelength of 415 nm on a Hitachi-F4500 fluorescence spectrophotometer.

2.4. Microbial growth assay

Effects of MRP on the microbial growth in fresh-cut TLL were also assayed. At a specified sampling time, 30 g sample were put into 225 ml sterile physiological saline and homogenized for 5 min, and then serial diluted (1:10). The serially diluted samples were surface-plated in triplicate onto plate count agar, incubated at 30 °C for 3 days for total aerobic mesophilic bacteria and also for total aerobic psychrotrophic bacteria incubated at 23 °C for 5 days. Lactic acid bacteria was detected by incubating at 30 °C for 4 days in de Man-Rogosa-Sharpe medium (0.15% sorbic acid) and Bangladeshired culture medium for mold and yeast was incubated for 3 days at 28 °C. The specifications proposed by Gómez-López, Ragaert, Jeyachchandran, Debevere, and Devlieghere (2008) were used to determine the end of the shelf-life from the microbiological point of view: 8 log cfu g^{-1} for aerobic mesophilic bacteria and aerobic psychrotrophic bacteria, 7 log cfu g^{-1} for lactic acid bacteria, and 5 log cfu g^{-1} for yeasts and molds.

2.5. Determination of DPPH radical scavenging activity and reducing power

The DPPH radical scavenging activity was determined according to the methods described by Kanatt et al. (2008). To 1 ml aliquot of appropriately diluted solution, 1 ml of ethanolic DPPH solution (0.2 mM) was added. The mixture was blended and left to stand at ambient temperature for 20 min. Reaction mixture containing 1 ml distilled water and 1 ml of ethanolic DPPH solution served as control. The absorbance of the solution was measured at 517 nm.

Reducing power was determined by ferricyanide method described by Yen and Duh (1993). Appropriately diluted sample (1 ml) was added to 2.5 ml of phosphate buffer (200 mM, pH 6.6) followed by 2.5 ml of 1% potassium ferricyanide. The reaction mixture was incubated for 20 min in a water bath at 50 °C. After incubation, 2.5 ml of 10% trichloroacetic acid was added, followed by centrifugation at 3000 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml distilled water and 1 ml of 0.1% ferric chloride. Absorbance of the resultant solution was measured at 700 nm. A high absorbance was indicative of strong reducing power.

2.6. Determination of copper-chelating properties

The copper-chelating property of MRP was determined according to the method described by Maillard, Billaud, Chow, Ordonaud, and Nicolas (2007). Tetramethylmurexide (TMM) (1 mmol/l) and $CuSO_4$ solutions (5 mmol/l) were prepared in 10 mmol/l hexamine-HCl buffer (pH 5.0) containing KCl (5 mmol/l). Absorbance readings at 480 nm (A_{480}) and 530 nm (A_{530}) were recorded. They corresponded to the maximum absorbance for (TMM-Cu) complex and for free TMM respectively.

2.7. Polyphenol oxidase (PPO) activity assay

Samples (10 g) were homogenized for 5 min in 500 mL of 0.05 M phosphate buffer (pH 7.0, 4 °C). The homogenates were immediately centrifuged at 15,000 × *g* for 20 min at 4 °C and the supernatants were collected. A spectrophotometric technique, adopted from Lee (2007), was used to measure PPO activity. 1 ml of PPO extracts was mixed with 4 ml of phosphate buffer and 1 ml of 0.5% (v/v) acetic acid solution (pH 5.0). After incubation for 5 min at 25 °C, 1 ml of 0.1 M catechol was added to the assay mixture. The reaction was run for 20 min and the absorbance of the solution was measured at 420 nm. To study the effects of DMWC or MRP on PPO activity, 1 ml of them were added instead of acetic acid solution.

2.8. Textural properties

Texture measurements were performed with a TA-XT2i Texture Analyser (Stable Micro Systems, London, UK) under optimal test conditions. The compression force at 30% strain was obtained by using a HDP/PFS probe. The samples were placed on the platform and measured with a 250 N cell at a deformation rate of 1 mm/s. The firmness of the samples was defined as the peak force at 30% strain.

2.9. Total soluble solids, ascorbic acid content and weight loss

Samples (10 g) from each treatment were crushed and homogenized with 100 ml of distilled water using a juice extractor (JYZ-E7, Joyoung Co. Ltd., China). The mixture was centrifuged at 5000 rpm for 15 min in a XZ-16T/TG16WS centrifuge (Changsha xiangzhi centrifuge instrument Co., Ltd., China), and the supernatants were for determination. Total soluble solids (TSS) were measured with a hand refractometer (Atago Co. Ltd., WYT-1, Tokyo, Japan). Ascorbic acid content (AAC) (mg/100 g) was determined by the Molybdenum blue method (Li, 2000). Weight loss was calculated by using following formulas: weight loss % = (IW − FW)/IW × 100, where IW represents initial weight; and FW represents weight after storage.

2.10. Color measurement

Color assessment was conducted using a Minolta Chroma Meter Model CR-300 (Minolta. Tokyo, Japan). The degree of browning was expressed as L^*, a^* and b^* value. The L^* scale ranges from 0 to 100 (white if $L^* = 100$, black if $L^* = 0$), which is a measure of brightness/whiteness. The parameter a^* scale ranges from $-a^*$ to $+a^*$ ($-a^*$ = green, $+a^*$ = red), and the b^* scale ranges from negative values for blue to positive values for yellow. Color (CIE L^*, a^*, b^*) was directly measured in a total of 3 locations on the surfaces of samples.

2.11. Statistical analysis

Experiments were performed in triplicate on different treatments. SPSS 19.0 software was used for data analysis. The mean values were calculated and reported as the mean ± S.D. ($n = 3$).

3. Results and discussion

3.1. Formation of chitosan-maltose MRP

As two kinds of different nonenzymatic browning, Maillard reaction results from condensation between nitrogen-containing compounds and the carbonyl group of reducing sugars, while caramelisation is caused by direct heating of carbohydrates. Fig. 1a and b shows the time course of absorbance at 280 nm and development of brown color as observed by absorbance at 420 nm. Maltose did not show any browning when heated alone, indicating that no disturbance was caused by Maillard reaction or caramelisation. Also, there was no significant color change when DMWC were heated alone. In contrast, an obvious change of absorbance was observed when mixtures were heated together. Generally, Maillard reaction follows a complex mechanism with three major stages (early, advanced and final stage), and the colored compounds are formed from Amadori intermediate or even melanoidins (Hofmann, Bors, & Stettmaier, 1999). Both browning and UV absorbance showed a slight induction period at the initial stage, and then came to a rapid growth, implying a progressive accumulation of MRP with time increasing. As shown in Fig. 1, the absorbance of LMRP at 280 nm and 420 nm was highest during 6 h of heating among the three kinds of reaction products. This means much more intermediate compounds of the Maillard reaction and darker color. After the induction period, UV–vis absorbance of HMRP were little higher than MMRP, although it was not significant ($p > 0.05$). Chevalier, Chobert, Popineau, Nicolas, and Haertle (2001) reported Maillard reaction rate is significantly influenced by the type of reducing sugar involved in the reaction. Generally, saccharides with shorter chains exhibited faster reaction rate than those with longer chains. Therefore, we concluded that the lower reaction rate of HMWC and MMWC may be due to their long polysaccharide chain than LMWC.

The Maillard reaction is also associated with the development of fluorescent compounds. Fluorophores formed during the Maillard reaction are the precursors of the brown pigments (Jing & Kitts, 2002). As shown in Fig. 1c, the fluorescence intensity of maltose was always weak as a function of reaction time. Due to their Maillard reaction, minimal increase of the intensity value was found for the heated DMWC. However, the intensity value was also low overall. When mixtures were heated together, the fluorescence intensity increased rapidly, showing fluorophores produced in the Maillard reaction between maltose and DMWC. The LMRP had the biggest fluorescence intensity, followed by HMRP and MMRP. Similar time-dependent increase in browning and florescence has been reported in sugar-amino acid model systems (Morales & Jimenez-Perez, 2001). All the results demonstrated the absorbance and fluorescent intensity of Maillard reaction had relationship to molecular weight of chitosan.

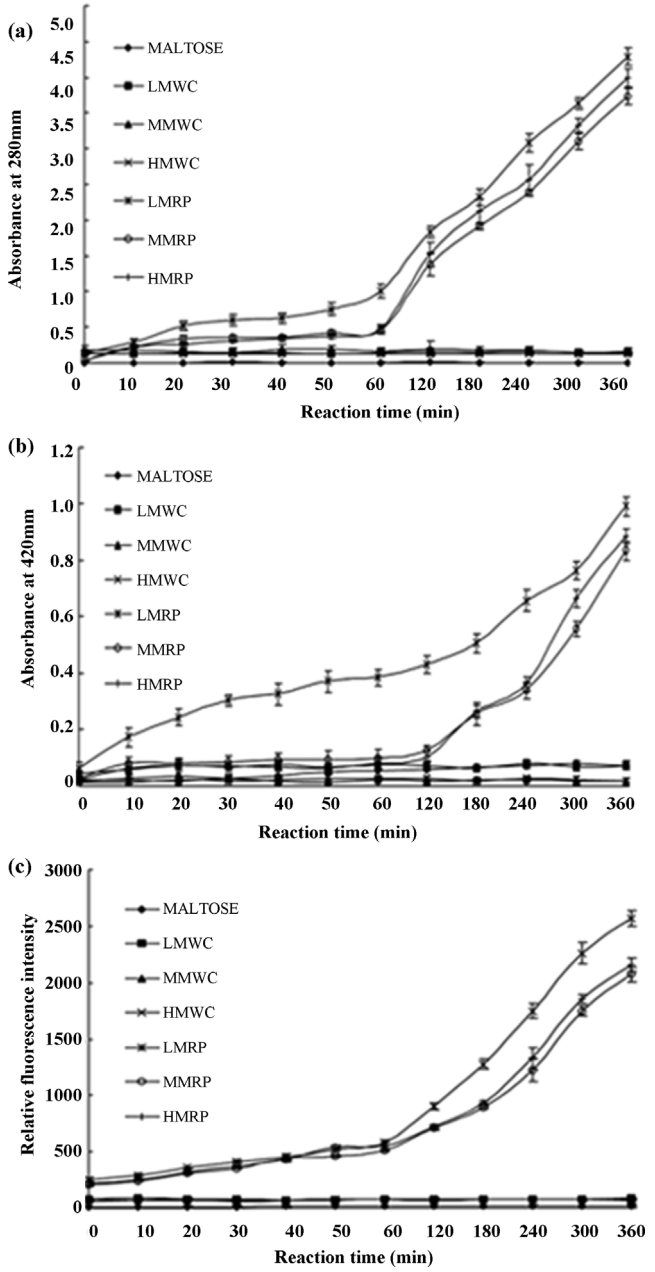

Fig. 1. Spectrophotometric changes of maltose, DMWC and MRP as a function of heating time (a) UV absorption at 280 nm; (b) browning at 420 nm; (c) fluorescent intensity at emission wavelength of 415 nm with excitation wavelength of 346 nm.

3.2. Effects of chitosan molecular weights on antioxidant activity of the MRP

The DMWC were found to influence the DPPH radical-scavenging activities and the reducing power of the heated solution. The heated mixtures were endowed with fine antioxidant activity against DPPH radical depending on heating time, while maltose and DMWC alone showed only minor scavenging potential (Fig. 2). Notably, the DPPH radical scavenging effect of the three kinds of MRP was changed during the heating. During the induction period, the LMRP exhibited the highest radical scavenging activity, followed by MMRP and HMRP. However, with the heating time prolongation, HMRP showed the highest radical scavenging activity. The increase in DPPH scavenging activity of HMRP may be due to the emergence of more intermediate compounds, which possessed good antioxidant activity. The IC_{50} of scavenging activity was

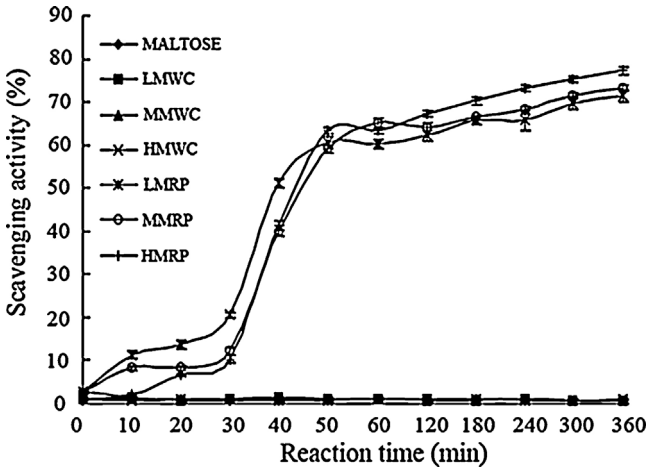

Fig. 2. DPPH free radical scavenging activity of maltose, DMWC and MRP as a function of heating time.

reached at 40, 44 and 46 min for LMRP, HMRP and MMRP, respectively. It can be described that 40 min heated mixture of 1 mg/ml HMWC and 1 mg/ml maltose (the conjugated was four-fold diluted) had good antioxidant activity, equal to the antioxidant ability of 2 mg/ml BHT. Ability of heat-induced MRP to scavenge DPPH radical has been reported in a lot of studies (Benjakul, Lertittikul, & Bauer, 2005; Jing & Kitts, 2002). Maillard et al. (2007) indicated that the MRP of glucose-cysteine had higher DPPH scavenging activities amongst the various glucose-amino acid mixtures, and proposed that this was probably due to the sulfhydryl group of cysteine. The better radical-scavenging activity of the conjugates can be attributed to the advanced Maillard reaction product melanoidins (Silvan, van de Lagemaat, Olano, & del Castillo, 2006), development of reductones (Singh & Rajini, 2004) and hydrogen transmission by OH or NH_2 groups in the pyranose rings (Xie, Xu, & Liu, 2001). According to present study, browning was not related to the free-radical scavenging activity of the MRP, therefore, could not act as a good indirect index to monitor the formation of MRP with free radical scavenging activity. This result was in accordance with the conclusion of Morales and Jimenez-Perez (2001).

The reducing activity of the MRP is shown in Fig. 3. DMWC-maltose conjugates showed an increasing reducing power when prolonging the heating time, but there was no remarkable reducing activity of DMWC or maltose alone, consistent with DPPH radical scavenging activity results above. And LMRP showed highest

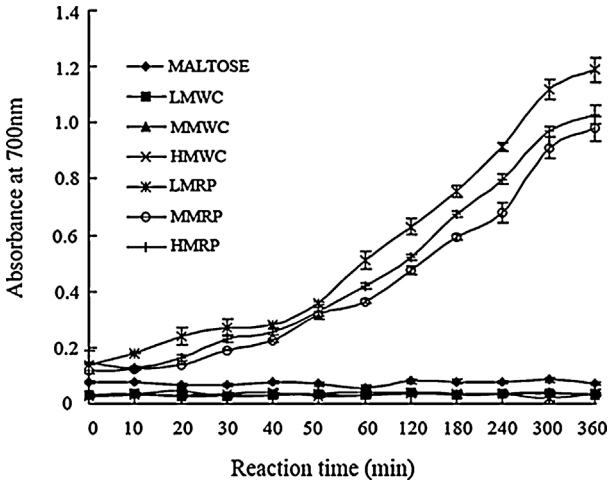

Fig. 3. Reducing power of maltose, DMWC and MRP as a function of heating time.

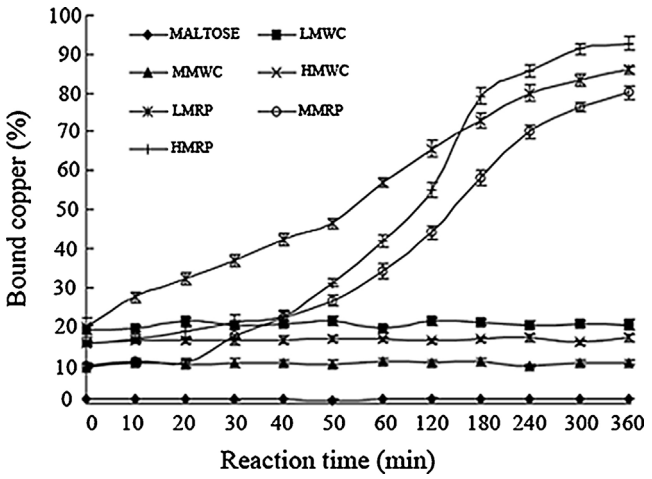

Fig. 4. Copper-chelating activity of maltose, DMWC and MRP as a function of heating time.

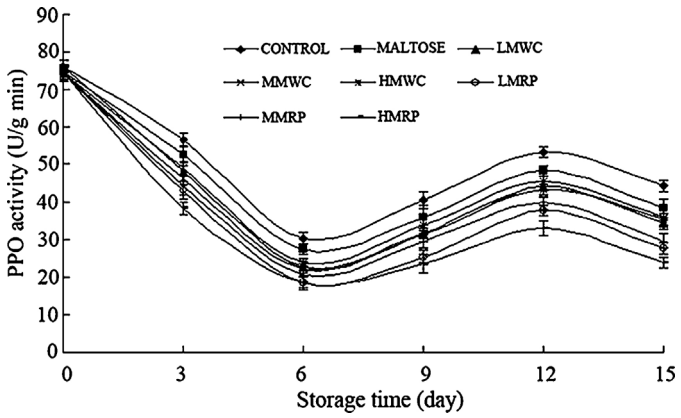

Fig. 5. Effects of control, maltose, DMWC and MRP on the activities of PPO of TLL during 15 days of storage at 20 °C.

reducing activity than any of the other two kinds of MRP during the reaction. From beginning to end, MMRP showed the lowest reducing activity of the three. Similarly, Yoshimura, Iijima, Watanabe, and Nakazawa (1997) found that the reducing activity also increased with increasing heating time of glucose-glycine mixtures.

Hwang, Shue, and Chang (2001) explained that maybe some of the Amadori products in the primary phase of Maillard reaction were responsible for its reducing activity. The compounds with reducing activity can be formed during thermolysis of Amadori products. Hydroxyl, pyrrole groups and intermediate reductone compounds of advanced MRP also contribute to reducing activity (Rao et al., 2011). The intermediate compounds of MRP were reported to be capable of donating hydrogen atoms (Yen & Hsieh, 1995). From the result, reducing activity was correlated with browning change as well as the formation of intermediate product.

3.3. *Effects of chitosan molecular weights on copper chelating properties of MRP*

Fig. 4 shows the copper chelating properties of MRP heated for different time. Different chitosan-maltose mixtures without heating exhibited relatively low chelating property. DMWC and maltose did not show significant variation ($p > 0.05$) when heated alone, however, much higher chelating power of the mixtures was observed with reaction time increasing. Chelating ability of MRP produced from DMWC was different from each other, and HMRP showed more pronounced chelating effect than the other two after heating for 2 h. For MRP prepared by heating for 5 h, HMRP exhibited 90% chelating activity, compared to 83% and 76% for LMRP and MMRP respectively. The result indicated that MRP had different chelating activities according to the heating time and MW of chitosan.

It has already been reported that melanoidins of MRP were able to bind metal cations such as Cu^{2+} (Bersuder, Hole, & Smith, 2001). And a key intermediate of early stage of Maillard reaction, which was Amadori rearrangement product, had chelating properties as well (O'Brien & Morrissey, 1997). Moreover, Seifert, Krause, Gloe, and Henle (2004) studied the complex formation of some MRP (N^{ε}-fructoselysine and N^{ε}-carboxymethyllysine) with copper and reported the formation of moderately stable copper complexes. Thus, it was possibly concluded that new copper-binding centers were formed on the heating reaction of DMWC with maltose. This suggested that there are much more copper-binding centers appeared in HMRP, which may be due to its long molecular chain. Therefore, HMRP showed stronger copper chelating ability that MMRP and LMRP after heating for 2 h.

3.4. *Effects of chitosan molecular weights on PPO inhibitory effects of MRP treated TLL*

Color is considered as a major determinant of TLL quality. Oxidation of phenolic compounds into quinones, via the polyphenol oxidase (PPO) that is inherent in tissue, is believed to be a major cause of browning of many fruits and vegetables (Deng, Wu, & Li, 2005). Inhibitory effects of MRP synthesized from heating DMWC with maltose for 5 h on PPO activity were exhibited in Fig. 5. There were no significant differences in the initial PPO activities among all the samples ($p > 0.05$). A fluctuation in activity during storage was detected, with a decrease in all samples in the first 6 days. PPO activities increased from day 6 to day 12 before they started to decrease again. PPO activities of the MRP-5 treated samples were lower than those of treated with maltose and DMWC and the control after day 12 ($p < 0.05$). The treatment with HMRP showed the lowest PPO activities among all the treatments at the end of the storage ($p < 0.05$).

It has already been shown that some MRP were able to generate PPO inhibitor compounds in fruits and vegetables. Roux, Billaud, Maraschin, Brun-Merimee, and Nicolas (2003) demonstrated that MRP from fructose, glucose and cysteine inhibited apple PPO activity. Also, Lee (2007) reported that MRP were recognized as strong banana PPO inhibitors. Additionally, Cheriot, Billaud, Pochtrager, Wagner, and Nicolas (2009) reported that MRP obtained by heating cysteine and hydroxymethylfurfural solution caused a strong inhibiting effect on eggplants PPO. From the present study, it can be seen that MRP enhanced the ability of PPO inhibition properties of chitosan.

PPO is a copper-containing enzyme that catalyzes both the *o*-hydroxylation of monophenols and the oxidation of *o*-diphenols (Xiao, Luo, Luo, & Wang, 2011). MRP could partly control PPO activity by interacting with the copper at the active site of the enzyme. Therefore, inhibitory effect on PPO activity of MRP on TLL may attribute to the copper chelating properties. And the results of PPO inhibition were consistent with the copper chelating of MRP in this study. In addition, the ability of inhibition PPO activity of MRP may be due to that coatings form a protective barrier on the surface of fresh products, reduce supply of oxygen, retard ripening and senescence, and consequently, delay browning of fruits and vegetables (Jiang & Li, 2001).

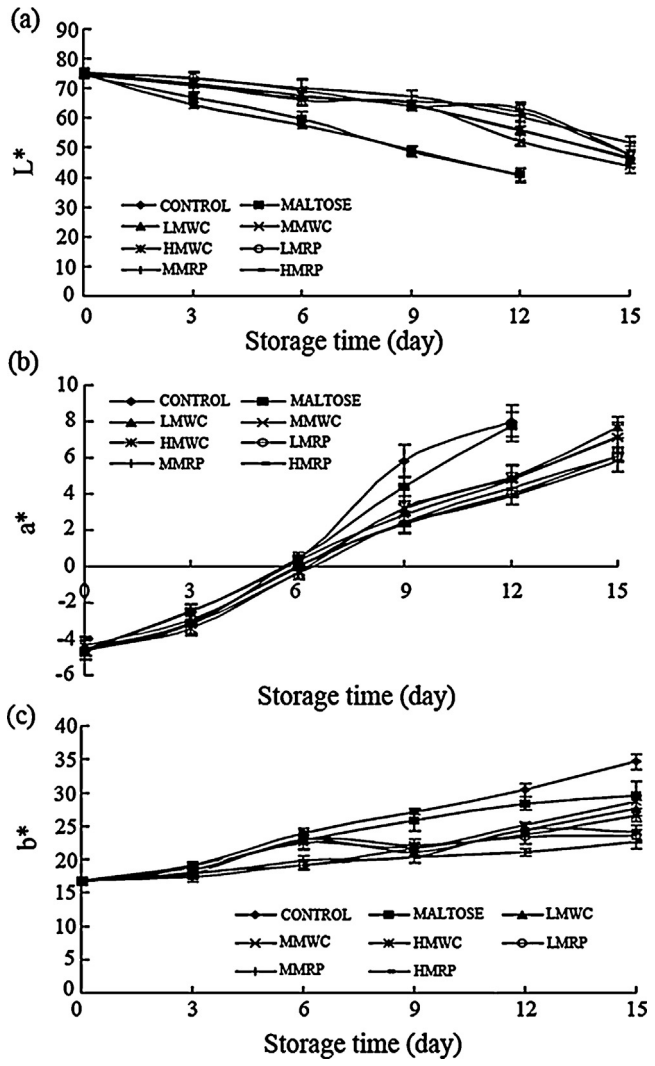

Fig. 6. Effects of control, maltose, DMWC and MRP treatments on L^*, a^* and b^* values of fresh-cut TLL during 15 days of storage at 20 °C.

3.5. Effects of chitosan molecular weights on color of MRP treated TLL

The effects of different treatments on L^*, a^* and b^* values of fresh-cut TLL were shown in Fig. 6. In general, the enzymatic browning of TLL during storage was accompanied by a decrease in lightness L^* and an increase in colorimetric a^* and b^*. The initial L^* values in all the samples were similar ($p > 0.05$). The L^* values in all treatments decreased during storage. For the treatment with maltose, L^* values were similar with those of control ($p > 0.05$), which dropped markedly compared to MRP and DMWC treated samples over 15 days of storage. The surfaces of the control and the maltose treated samples were not acceptable after 12 days storage (no need to test). L^* values of MRP treated samples were higher than those of the control and DMWC treated samples. The higher the L^* value of fresh-cut TLL, the brighter was the surface. HMRP was most effective for inhibiting the browning on the surface of fresh-cut TLL, which showed the slowest decreasing trend during storage.

Values of a^* indicate the color change from green to red during storage, and the high a^* values mean browning of samples. As shown in Fig. 6b, a^* values of TLL samples of all treatments tended to increase with increasing storage time, showing that fresh-cut TLL turned browning during storage. And a^* values of the controls increased most quickly compared to those of other treatments. Among the DMWC and MRP treated samples, a^* values of TLL treated with HMRP were significantly lower than the others after 15 days storage, which suggesting that HMRP had the potential to inhibit the discoloration of fresh-cut TLL. The b^* value was used to be a measurement of yellowness. The more increasing in b^* value, the more yellow color formed on the fresh-cut TLL samples. Similarly to a^* value, samples treated with MRP showed slower increasing rate of b^* value, showing ability in inhibiting yellow color on the TLL surface. A recent study also proposed that chitosan-xylose Maillard reaction products could effectively inhibit the darkening of semi-dried noodles (Zhu et al., 2013).

These results suggest that HMRP had the outstanding potential to inhibit the discoloration of fresh-cut TLL. And both of a^* and b^* values were consistent with the corresponding L^* values. The change in color was due to oxidative reactions of phenolic compounds by polyphenol oxidase and the reaction products, oquinones, to various polymerized products (Nicoli, Elizalbe, Piotti, & Lerici, 1991). Therefore, MRP inhibitory effect on PPO activity as above mentioned might account for this phenomenon.

3.6. Effects of chitosan molecular weights on microbial growth of MRP treated TLL

Mesophilic bacteria, psychrotrophic bacteria, yeasts and molds and lactic acid bacteria predominated during storage in all treated TLL samples. To ascertain the antimicrobial and shelf-life extending properties of MRP on fresh-cut TLL, samples were treated with MRP-5. MRP-5 markedly inhibited growth of microorganisms tested, however, the inhibitory effects differed due to the molecular weight of chitosan and the type of microorganisms. The initial populations of aerobic mesophilic bacteria and aerobic psychrotrophic bacteria in fresh-cut TLL were approximately 3.4 log cfu g^{-1} and 2.4 log cfu g^{-1}, respectively. Lactic acid bacteria (1.75 log cfu g^{-1}), yeasts and molds (2.7 log cfu g^{-1}) were also measured (Table 1). In spite of the reduction achieved after MRP treatment, the microbial numbers increased with increasing storage time at 20 °C. It is evident from this study that MRP coating was more effective in inhibiting microbial counts than other treatments ($p < 0.05$). For different microbial group, the effect of inhibiting microbial growth of TLL was different by MRP. The populations of psychrotrophic bacteria increased to unacceptable levels (>8 log cfu g^{-1}) in the control after 12 days, but kept acceptable in MRP treated samples till the end of the storage ($p < 0.05$). Similarly, MRP treatment also delayed increasing of aerobic mesophilic bacteria to unacceptable levels for 3 days.

The populations of lactic acid bacteria in control reached 7 log cfu g^{-1} after 12 days but maintained acceptable in MRP treated samples during the whole storage ($p < 0.05$). Yeasts and molds counts in the control became unacceptable (>5 log cfu g^{-1}) from day 9, while MRP treated samples remained acceptable over 9 days ($p < 0.05$). At day 15, HMRP treated samples showed the lowest microbial counts of psychrotrophic bacteria and aerobic mesophilic bacteria. However, it was LMRP when it came to lactic acid bacteria, yeasts and molds respectively.

Kanatt et al. (2008) have reported the antimicrobial activity of chitosan-glucose complex was similar to chitosan against *E. coli*, *Pseudomonas*, *S. aureus* and *B. cereus*. However, in the present experiment, chitosan-maltose complex exhibited superior antimicrobial activity to chitosan. Therefore, the MRP-5 enriched TLL more resistant to microbial growth than the control, maltose and DMWC. The results suggested TLL treated with MRP did not favor the development of tested bacteria. Therefore, microbial degradation resulting in changes such as browning and softening was delayed in MRP treated samples to some extent.

Table 1
Effects of maltose, DMWC and MRP treatment on microbial growth (log cfu g^{-1}) of fresh-cut TLL during 15 days of storage at 20 °C.

Storage time (day)	Control	Maltose	LMWC	MMWC	HMWC	LMRP	MMRP	HMRP
Aerobic mesophilic bacteria								
0	3.39 ± 0.04a	3.35 ± 0.05a	3.40 ± 0.03a	3.40 ± 0.05a	3.42 ± 0.03a	3.42 ± 0.03a	3.42 ± 0.04a	3.36 ± 0.08a
3	5.24 ± 0.05d	5.20 ± 0.07 cd	5.09 ± 0.07bc	5.11 ± 0.06bcd	5.06 ± 0.03b	4.91 ± 0.03a	5.11 ± 0.08bcd	5.03 ± 0.15ab
6	6.71 ± 0.10c	6.75 ± 0.06c	6.11 ± 0.09b	6.15 ± 0.10b	6.05 ± 0.09b	5.84 ± 0.08a	5.75 ± 0.07a	5.89 ± 0.09a
9	8.71 ± 0.10e	8.74 ± 0.08e	7.70 ± 0.12c	7.80 ± 0.04 cd	7.90 ± 0.09d	7.18 ± 0.08b	7.10 ± 0.14ab	6.93 ± 0.10a
12	ND	ND	8.56 ± 0.13c	8.45 ± 0.08c	8.37 ± 0.11c	7.71 ± 0.14b	7.56 ± 0.10b	7.36 ± 0.11a
15	ND	ND	ND	ND	ND	8.71 ± 0.20b	8.50 ± 0.17ab	8.23 ± 0.13a
Psychrotrophic bacteria								
0	2.40 ± 0.04a	2.40 ± 0.06a	2.42 ± 0.04a	2.42 ± 0.04a	2.37 ± 0.06a	2.42 ± 0.03a	2.41 ± 0.07a	2.40 ± 0.08a
3	3.53 ± 0.07a	4.05 ± 0.10bc	4.03 ± 0.08bc	4.11 ± 0.06c	3.98 ± 0.09bc	3.88 ± 0.08b	4.06 ± 0.10c	4.16 ± 0.15c
6	5.63 ± 0.09e	5.68 ± 0.10e	5.07 ± 0.06 cd	5.01 ± 0.08c	5.18 ± 0.06d	4.74 ± 0.16b	4.32 ± 0.12a	4.96 ± 0.09c
9	7.68 ± 0.06e	7.81 ± 0.07e	6.59 ± 0.13c	6.86 ± 0.09d	6.66 ± 0.09c	6.28 ± 0.06b	5.83 ± 0.12a	6.13 ± 0.10b
12	8.46 ± 0.13f	8.64 ± 0.07f	7.39 ± 0.06d	7.65 ± 0.16e	7.39 ± 0.13d	6.84 ± 0.10c	6.36 ± 0.11a	6.60 ± 0.16b
15	ND	ND	8.19 ± 0.15c	8.33 ± 0.09c	8.23 ± 0.11c	7.81 ± 0.16b	7.20 ± 0.17a	7.25 ± 0.11a
Lactic acid bacteria								
0	1.76 ± 0.04a	1.76 ± 0.04a	1.75 ± 0.04a	1.77 ± 0.05a	1.76 ± 0.08a	1.75 ± 0.04a	1.75 ± 0.04a	1.75 ± 0.04a
3	2.71 ± 0.02d	2.64 ± 0.03d	2.18 ± 0.16bc	1.98 ± 0.09a	2.21 ± 0.09c	2.12 ± 0.10abc	2.15 ± 0.11abc	2.02 ± 0.08ab
6	3.79 ± 0.02e	3.65 ± 0.07d	2.94 ± 0.08b	3.12 ± 0.08c	3.26 ± 0.09c	2.86 ± 0.08ab	2.92 ± 0.05b	2.76 ± 0.10a
9	5.19 ± 0.05e	5.22 ± 0.10e	4.23 ± 0.10 cd	4.36 ± 0.04d	4.13 ± 0.09c	3.91 ± 0.12b	3.82 ± 0.06b	3.56 ± 0.06a
12	7.27 ± 0.04e	7.33 ± 0.09e	6.16 ± 0.13d	6.07 ± 0.05 cd	5.94 ± 0.08bc	5.86 ± 0.09b	5.49 ± 0.14a	5.92 ± 0.04bc
15	ND	ND	7.12 ± 0.15b	7.38 ± 0.18c	7.25 ± 0.07bc	6.76 ± 0.10a	6.90 ± 0.07a	6.81 ± 0.11a
Yeasts and molds								
0	2.68 ± 0.02a	2.70 ± 0.02a	2.69 ± 0.03a	2.71 ± 0.03a	2.72 ± 0.06a	2.70 ± 0.02a	2.72 ± 0.04a	2.71 ± 0.07a
3	3.36 ± 0.04c	3.34 ± 0.02c	3.01 ± 0.13b	3.17 ± 0.06bc	3.10 ± 0.08b	2.99 ± 0.17b	3.04 ± 0.13b	2.79 ± 0.11a
6	4.48 ± 0.04d	4.52 ± 0.03d	4.01 ± 0.12abc	3.88 ± 0.07a	4.14 ± 0.11c	3.88 ± 0.04a	4.03 ± 0.09bc	3.92 ± 0.04ab
9	5.84 ± 0.06c	5.88 ± 0.04c	4.65 ± 0.20b	4.71 ± 0.15b	4.75 ± 0.08b	4.24 ± 0.21a	4.15 ± 0.12a	4.31 ± 0.11a
12	ND	ND	5.79 ± 0.18b	5.92 ± 0.08b	5.86 ± 0.13b	4.79 ± 0.18a	4.76 ± 0.19a	4.66 ± 0.10a
15	ND	ND	ND	ND	ND	5.27 ± 0.12a	5.51 ± 0.12a	5.37 ± 0.14a

ND, not determined.
Values are shown as mean ± standard deviation. Means followed by the same letter in the same row are not significantly different ($p > 0.05$).

3.7. Effects of chitosan molecular weights on weight loss of MRP treated TLL

A large increase in water loss occurred with control as shown in Table 2. Compared with the control samples, the coated TLL showed a significantly ($p < 0.05$) reduced weight loss after the third day. On day 1, the HMRP showed the lowest weight loss (0.52%), followed by LMRP (0.57%) and MMRP (0.6%). After 15 days of storage, the weight loss in the LMRP treated sample was significantly ($p < 0.05$) lower than that in the other coated TLL. And the TLL coated with LMRP showed the lowest weight loss of 13.2%, as compared to 21.9%, 19.4% and 16% weight loss in control, maltose and LMWC coated samples, respectively.

In this study, the chitosan and MRP coating significantly reduced the weight loss of TLL at 20 °C compared with uncoated fruit, and therefore delayed fruit shriveling and quality deterioration. Similar excellent effects of the chitosan treatments in weight loss have been observed in other fresh-cut vegetables and fruits, including Chinese water chestnut (Pen & Jiang, 2003), lotus root (Xing et al., 2010) and pears (Xiao et al., 2011). According to Watada and Qui (1999), the loss of water is a natural process of the catabolism of fresh-cut vegetables, and is attributed to the respiration and other senescence-related metabolic processes during storage. Therefore, MRP coating possibly delayed the migration of moisture into the environment, and decreased respiration consumption, which resulting in decreasing weight loss.

In the present study, MRP was the most effective in reducing weight loss. The mechanism of chitosan-maltose complex inhibits the increase of weight loss is not clear. Similarly, chitosan-glucose complex was found to be more effective to inhibit the increase of weight loss of table grapes (Gao et al., 2013) and shiitake mushroom (Jiang et al., 2012) under cold storage, when compared with chitosan or glucose alone.

3.8. Effects of chitosan molecular weights on firmness of MRP treated TLL

Firmness values of all samples decreased with increased storage time to varying degrees (Table 2), but the control group decreased faster compared with those treated with MRP and DMWC. All the samples presented similar initial firmness values ($p > 0.05$). For 15 days storage, firmness in the control samples decreased by 70.2%, whereas samples coated with maltose, HMWC, or MMRP showed the values of 60.9%, 48.3% and 42.6%, respectively. It was shown that TLL with MMRP was significantly ($p < 0.05$) highest in firmness.

It has been reported that the textural changes of fruits and vegetables were influenced by cell wall-degrading enzymes, such as pectin methylesterase (PME), polygalacturonas (PG), pectinesterase (PE) and cellulase (Cx). Protopectin are catalyzed by pectinases to form water-soluble pectin. When pectin enters the cell sap under osmotic pressure, it forms a gel, causing fruit softening (Deng et al., 2005). As for TLL an unacceptable consequence of cutting is softening, which mainly owing to enzymatic hydrolysis of cell wall pectic substances and the thinning of cell walls. Therefore, the results indicated that the ability of MRP and DMCW, especially for MMRP, to maintain firmness was acquired by inhibiting the activities of aforementioned enzymes. Additionally, softening is often related to loss of turgor pressure in the cells reduced by water loss (Lohani, Trivedi, & Nath, 2004). Therefore, MRP coatings may maintain firmness of TLL by reducing water loss. This result was consistent with the weight loss of TLL.

3.9. Effects of chitosan molecular weights on TSS and AAC of MRP treated TLL

The TSS and ascorbic acid levels decreased in all samples after 15 days storage at 20 °C, however, the use of DMWC or MRP

Table 2
Effects of maltose, DMWC and MRP treatment on weight loss, firmness, TSS and AAC of fresh-cut TLL during 15 days of storage at 20 °C.

Storage time (day)	Control	Maltose	LMWC	MMWC	HMWC	LMRP	MMRP	HMRP
Weight loss (%)								
0	0	0	0	0	0	0	0	0
3	1.07 ± 0.21d	0.90 ± 0.18 cd	0.84 ± 0.06c	0.75 ± 0.06bc	0.89 ± 0.07 cd	0.57 ± 0.03ab	0.60 ± 0.06ab	0.52 ± 0.10a
6	4.07 ± 0.21c	3.72 ± 0.08bc	3.11 ± 0.16a	3.50 ± 0.20b	3.87 ± 0.38bc	2.87 ± 0.25a	3.07 ± 0.15a	2.97 ± 0.12a
9	8.13 ± 0.32c	7.53 ± 0.25c	6.73 ± 0.21b	6.47 ± 0.35b	6.13 ± 0.35b	4.87 ± 0.71a	4.73 ± 0.24a	5.03 ± 0.15a
12	13.5 ± 0.50d	12.03 ± 0.57c	10.77 ± 0.67ab	11.43 ± 1.20bc	12.10 ± 0.56c	9.77 ± 0.67a	10.00 ± 0.46a	9.87 ± 0.65a
15	21.9 ± 0.7f	19.4 ± 0.86e	16.03 ± 0.38bc	16.67 ± 0.60 cd	17.67 ± 0.40d	13.23 ± 0.81a	15.10 ± 0.20b	15.47 ± 0.60b
Firmness (N)								
0	20.93 ± 0.80a	21.1 ± 0.75a	20.9 ± 0.66a	21.03 ± 0.74a	21.1 ± 0.56a	21.17 ± 0.57a	20.93 ± 0.70a	21.06 ± 0.59a
3	18.03 ± 0.45a	18.23 ± 0.31a	18.9 ± 0.87a	17.67 ± 0.76a	17.93 ± 0.51a	18.03 ± 0.76a	20.43 ± 0.57b	18.27 ± 0.78a
6	16.33 ± 0.65a	17.07 ± 0.32ab	18.00 ± 0.44bc	18.63 ± 0.61 cd	17.93 ± 0.57bc	18.30 ± 0.79 cd	19.30 ± 0.79d	18.10 ± 0.62bc
9	13.27 ± 0.70a	14.73 ± 0.61b	16.83 ± 0.40 cd	17.57 ± 0.47de	17.80 ± 0.35de	16.13 ± 0.50c	18.10 ± 0.72e	16.23 ± 0.65c
12	8.67 ± 0.35a	10.74 ± 0.51b	11.67 ± 0.72bcd	11.87 ± 0.91bcd	11.33 ± 0.74bc	12.53 ± 0.65 cd	16.07 ± 0.67e	12.83 ± 0.58d
15	6.23 ± 0.32a	8.23 ± 0.40b	9.80 ± 0.69c	10.27 ± 0.32c	10.87 ± 0.45 cd	10.6 ± 1.04 cd	12.03 ± 0.35e	11.40 ± 0.75de
TSS (%)								
0	2.61 ± 0.04a	2.59 ± 0.04a	2.59 ± 0.04a	2.58 ± 0.04a	2.60 ± 0.04a	2.59 ± 0.05a	2.59 ± 0.06a	2.60 ± 0.06a
3	1.75 ± 0.05a	1.80 ± 0.05ab	2.00 ± 0.08 cd	1.93 ± 0.04c	1.90 ± 0.06bc	2.13 ± 0.05e	2.08 ± 0.06de	2.14 ± 0.08e
6	1.80 ± 0.04a	1.83 ± 0.02a	2.10 ± 0.04c	2.05 ± 0.09bc	2.11 ± 0.06c	2.02 ± 0.09bc	1.96 ± 0.04b	2.10 ± 0.12c
9	1.78 ± 0.05a	1.83 ± 0.03a	2.13 ± 0.07b	2.09 ± 0.08b	2.11 ± 0.10b	2.16 ± 0.05b	2.10 ± 0.04b	2.19 ± 0.12b
12	1.23 ± 0.07a	1.31 ± 0.04a	1.48 ± 0.09b	1.62 ± 0.07bc	1.65 ± 0.06c	1.92 ± 0.12d	1.91 ± 0.08d	2.00 ± 0.10d
15	0.83 ± 0.07a	0.96 ± 0.14a	1.33 ± 0.08b	1.49 ± 0.06c	1.51 ± 0.07c	1.78 ± 0.06d	1.82 ± 0.08d	1.91 ± 0.08d
AAC (mg/100 g)								
0	8.56 ± 0.04a	8.56 ± 0.04a	8.56 ± 0.04a	8.56 ± 0.04a	8.56 ± 0.04a	8.56 ± 0.04a	8.56 ± 0.04a	8.56 ± 0.04a
3	6.54 ± 0.06a	6.61 ± 0.10a	6.67 ± 0.10a	6.63 ± 0.09a	6.57 ± 0.16a	6.90 ± 0.04b	6.87 ± 0.02b	7.00 ± 0.12b
6	5.19 ± 0.08a	5.29 ± 0.03ab	5.30 ± 0.10abc	5.37 ± 0.07bc	5.41 ± 0.06c	5.96 ± 0.06d	6.02 ± 0.08d	6.05 ± 0.05d
9	3.42 ± 0.09a	3.47 ± 0.04ab	3.60 ± 0.10bc	3.50 ± 0.09ab	3.66 ± 0.07c	3.92 ± 0.07de	3.82 ± 0.09d	3.99 ± 0.07e
12	2.16 ± 0.07a	2.26 ± 0.04ab	2.36 ± 0.04bc	2.46 ± 0.09c	2.59 ± 0.04d	2.92 ± 0.06e	2.93 ± 0.10e	3.03 ± 0.10e
15	1.32 ± 0.11a	1.40 ± 0.06ab	1.48 ± 0.04b	1.51 ± 0.04b	1.65 ± 0.12c	2.06 ± 0.05d	2.01 ± 0.07d	2.13 ± 0.05d

Values are shown as mean ± standard deviation. Means followed by the same letter in the same row are not significantly different ($p > 0.05$).

significantly reduced the loss of these two kinds of nutritional components in TLL samples (Table 2). No significant difference was observed between control and maltose treatments during the storage ($p > 0.05$). There was a fluctuation in TSS during storage, with a decrease in all samples in the first 3 days. TSS increased from day 3 to day 9 before they began to decrease. TSS of the control was lower than those of DMWC or MRP treated samples. By day 15, the TSS content in the HMCW and HMRP treated fruit was approximately 1.82 and 2.3 times as much as that in the control.

Similar to TSS, the level of AAC was lower in the control than in the coated samples during storage. After 15 days of storage, ascorbic acid retention of TLL treated with maltose, HMWC and HMRP coating was 1.4, 1.65 and 2.13 mg/100 g respectively, whereas control samples maintained 1.32 mg/100 g of AAC.

Our study revealed that TLL treated with HMRP had the best effect on retarding consumption of important nutritional components, such as soluble solids and ascorbic acid. The reason may be due to that HMRP had a better potential to limit respiration metabolism and fungal growth samples than DMWC. It has been suggested that chitosan could play as an abiotic elicitor to generate reactive oxygen species, which are scavenged by ascorbic acid. MRP had the ability to inhibit ascorbic acid loss, due to the protection effected by its superior antioxidant activity, as compared to chitosan (Kanatt et al., 2008).

4. Conclusions

Our results demonstrated chitosan-maltose MRP were endowed with better antioxidant and antimicrobial activities than chitosan alone, and the activities differed depending on chitosan molecular weight. Chitosan-maltose MRP could be used as a promising vegetable preservative. And the quality and shelf-life of MRP treated TLL varied owing to different molecular weight of citosan.

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