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Functional properties of chitosan–xylose Maillard reaction products and their application to semi-dried noodle

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ABSTRACT

The objective of this research was to evaluate the antimicrobial, antioxidant and darkening inhibitory activities of Maillard reaction products (MRPs) prepared from chitosan and xylose, and their effects on the preservation and quality of semi-dried noodles. The development of brown color and UV absorbance changes indicated the proceeding of Maillard reaction. The antimicrobial activity, reducing power, DPPH• radical scavenging activity, copper-chelating activity and PPO inhibitory effects of chitosan–xylose conjugates increased with the reaction. Addition of MRPs could improve the textural and cooking quality of semi-dried noodles. Incorporation of 0.35% MRPs (6 h) into semi-dried noodle resulted in an extension of shelf life for more than 7 days than the control noodles (at room temperature). The discoloration caused by the addition of MRPs was limited, and MRPs could inhibit darkening of the noodle effectively. Results suggested that the MRPs could be used as a novel promising preservative for semi-dried noodles.

1. Introduction

Noodles have been the staple foods for many countries in Asia since ancient time. Nowadays, their world consumption has increased, due to the ease of transportation, handling, cooking and preparation (Li et al., 2012).

Semi-dried noodle, a newly developed instant noodle product, is generally produced by undergoing a high-temperature short-time (HTST) dehydration process to get a final water content of 20–25%. It is getting increasingly popular to consumers due to the better taste and flavor compared to dry noodles.

However, despite the dehydration treatment, semi-dried noodles are vulnerable to microbial contamination for their relatively high moisture content and abundant nutrient substances, especially in summer days. The survived microorganisms will proliferate immediately if not inhibited by particular technologies, which limits the distribution of semi-dried noodles. Thus, due to the industry demands, interest has been generated in searching for preservation methods and technologies for these products (Xu, Hall, Wolf-Hall, & Manthey, 2008). At present, most studies turn to use various chemical preservatives, such as potassium sorbate, sodium dehydroacetate and calcium propionate. However, while producers are interested in extending their shelf life, the consumer is increasingly calling for more "green" food (Diez, Santos, Jaime, & Rovira, 2009). In addition, most of these chemical preservatives may deteriorate the flavor and texture of the final products. These situations have led to the current search for natural and versatile antimicrobial agents which have a natural image.

Chitosan manufactured from chitin is the linear and partly acetylated (1–4)-2-amino-2-deoxy-β-D-glucan (Muzzarelli, 1977; Muzzarelli et al., 2012). As a kind of polysaccharides, chitosans were tested as a dietary supplement for their favorable biological properties, low toxicity and high susceptibility to biodegradation (Muzzarelli, 1996; Muzzarelli & Muzzarelli, 2006). It exhibits antimicrobial activity and has therefore attracted tremendous attention as a potential food preservative of natural source (Holappa et al., 2006). However, chitosan is insoluble in the neutral or alkaline range. Further more, the solution of chitosan is quite viscous even at low concentrations (Jin, Wang, & Bai, 2009). These restricted its application in a commercial context.

Maillard reaction, which is also called nonenzymatic browning reaction, is a reaction between reducing compounds and amino groups. In addition to contributing to the formation of a specific color and flavor, many studies have reported that Maillard reaction products (MRPs) bear excellent antioxidative and antimicrobial activity as well (Jing & Kitts, 2002; Kim & Lee, 2003; Martins, Jongen, & Van Boekel, 2000). Due to its amino group, chitosan can be participant in the Maillard reaction to react with carbonyl group of a reducing sugar. It has been reported that the MRPs produced by heating chitosan with glucose showed significantly higher antimicrobial and antioxidant activity than chitosan or glucose alone (Kanatt, Chander, & Sharma, 2008). Thus Maillard reaction is a promising and effective way to produce novel preservative.

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In this study, chitosan–xylose Maillard reaction products were synthesized to take advantage of their antimicrobial activity; their influence on the quality and shelf-life of semi-dried noodle were investigated.

2. Materials and methods

2.1. Materials

Chitosan (deacetylation degree 85%, MW 400 kDa) was obtained from Golden-Shell Pharmaceutical Co., Ltd. (Zhejiang, China). D-Xylose was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) was purchased from Sigma–Aldrich (St. Louis, MO). Semi-dried noodle (water content 22.34 ± 0.47%, pH 6.36 ± 0.03) was prepared in our laboratory by using a Kitchen Aid mixer (Kitchen Aid, St. Joseph, MI, USA), a laboratory sheeting machine (Mode JMTD-168/140, Beijing, China) and an UV-equipped microwave oven (Mode JHWB-MF4, Guangzhou, China).

2.2. Preparation of chitosan–xylose conjugates

Chitosan was dissolved in 0.5% (v/v) acetic acid solution to reach a concentration of 1% (w/v) and then 1% (w/v) xylose was dissolved in the chitosan solution. The pH value of the solution was adjusted to 5.0 using 1 M sodium hydroxide. The mixtures were then heated in a water bath at 80 °C for up to 8 h. Samples were withdrawn at given time intervals, and cooled in an ice-water bath.

2.3. Spectrophotometric analysis of MRPs

Browning and UV absorbance of the conjugates were measured according to Li, Shi, Wang, and Du (2011). The samples were appropriately diluted with 0.5% (v/v) acetic acid solution. UV absorbance and browning intensity were measured at 280 nm and 420 nm respectively.

2.4. Determination of antimicrobial activity

The antimicrobial activities of MRPs incubated for different times against *Staphylococcus aureus*, *Bacillius licheniformis* or *Aspergillus candidus* were determined using the minimum inhibitory concentration (MIC) method. These strains were identified as the most common spoilage organisms in semi-dried noodle products according to our previous study. During the determination, 0.1 ml diluted cell suspension, with a bacteria content of approximately 10^6 CFU/ml, was added to a tube of 5 ml broth medium containing an appropriate concentration of MRPs. The tubes were incubated for 48 h at 37 °C for all strains with the exception of the *A. candidus* tubes which were incubated for 120 h at 28 °C. The MIC was defined as the lowest concentration of MRPs required to completely inhibit microorganism growth.

2.5. Determination of reducing power and DPPH• radical scavenging activity

The reducing power and DPPH• radical scavenging activity were determined according to the methods described by Ferreira, Baptista, Vilas-Boas, and Barros (2007). 1.0 ml of the diluted sample was added into 4.0 ml of 0.1 mM DPPH• in ethanol and the solution was mixed immediately. After incubation in the dark for 20 min, the absorbance was measured at 517 nm. Percent of DPPH scavenging activity was calculated as follows:

$$\text{Scavenging activity}\ (\%) = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}}\right] \times 100 \qquad (1)$$

A_{sample} was the absorbance of the sample; A_{control} was the absorbance of 0.5% (v/v) acetic acid solution (pH = 5.0) reacted at the same condition as the sample.

For reducing power, 1 ml of appropriately diluted sample was mixed with 1 ml of 0.2 M sodium phosphate (pH 6.6) and 1 ml of 1% (w/v) potassium ferricyanide. After incubation at 50 °C for 20 min, 2.5 ml of 10% (w/v) trifluoroacetate was added to the solution and the mixture was centrifuged at 3000 r/min for 10 min. 2 ml of the supernatant was mixed with 2 ml distilled water and 1 ml 0.1% (w/v) ferric chloride. After incubation for 10 min, the absorbance was measured at 700 nm.

2.6. Determination of copper-chelating properties

The copper-chelating property of MRPs was determined according to the method described by Matmaroh, Benjakul, and Tanaka (2006).

1 ml of $CuSO_4$ solution in different concentration (0–0.1 mM) was mixed with 4.0 ml of hexamine buffer (10 mM hexamethylene tetramine, 10 mM KCl, pH 5.0) and 0.2 ml of 1 mM tetramethyl murexide (TMM). $A_{460\,\text{nm}}$ and $A_{530\,\text{nm}}$ of the solution were determined respectively. The absorbance ratio $A_{460\,\text{nm}}/A_{530\,\text{nm}}$ was plotted against the amount of $CuSO_4$ to obtain the standard curve.

0.5 ml of diluted (20 fold) chitosan or MRPs was mixed with 1.5 ml of hexamine buffer and 0.5 ml of 2 mM $CuSO_4$. The mixture was incubated for 60 min at room temperature. Then 0.2 ml of the mixture was diluted to 5.0 ml with hexamine buffer. 0.2 ml of 1 mM TMM was added into the solution. After incubated for 10 min, $A_{460\,\text{nm}}$ and $A_{530\,\text{nm}}$ were determined respectively. The ratio $A_{460\,\text{nm}}/A_{530\,\text{nm}}$ was calculated to obtain the amount of the free copper remained in the solution according to the standard curve.

2.7. Polyphenol oxidase (PPO) activity assay

The PPO for test was extracted from the wheat flour used to make semi-dried noodles, which was similar to previously published procedures (Fuerst, Xu, & Beecher, 2008). 2 g of flour was incubated in 10 ml of extraction buffer (0.1 M phosphate–citric acid buffer, pH 5.6) in a 15 ml centrifuge tube and shaked at 4 °C for 12 h. The sample was then centrifuged at 12,000 rpm for 15 min at 4 °C and the supernatant was used as the crude extracts of PPO.

The PPO activity was determined according to the method described by Lee (2007). 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 3 min at 37 °C, 1 ml of 0.1 M catechol was added to the mixture to initiate the reaction. The reaction was run for 20 min and the absorbance of the solution was measured at 420 nm. One unit was defined as the change in 0.001 U of $A_{420\,\text{nm}}$/min g. To study the effects of chitosan or MRPs on PPO activity, 1 ml of them were added instead of acetic acid solution.

2.8. Cooking loss determination

The cooking loss, that is, the amount of solid substance lost to cooking water, was determined as described by AACC Method 66-50 (AACC, 2000). A 25 g sample of noodle was placed into 400 ml of boiling distilled water until the optimal cooking time. Cooking water was collected in a 500 ml volumetric flask and made to volume, then 50 ml was taken into a 100 ml beaker pre-dried to a constant weight, after that, placed the beaker into an air oven at 105 °C and evaporated to dryness. The residue was weighed and reported as a percentage of the starting material (calculated by dry basis).

For each optimal cooking time and cooking loss value, three determinations were performed to obtain the mean value.

2.9. Textural properties

Textural properties of cooked noodles were measured using a TA-XT2i Texture Analyser (Stable Micro Systems, London, UK) under optimal test conditions. Fresh noodles were first cut into strands of 20 mm and cooked to the optimal cooking time. Measurements were carried out at room temperature exactly 10 min after cooking. Tensile force was determined by a A/SPR probe in the mode of "Measure Force in Tension", maximum shearing force was measured using a A/LKD probe in the mode of "Measure Force in Compression", while hardness, springiness, and chewiness were tested by a HDP/PFS probe and calculated based on texture profile analysis (TPA) according to the description of Wu and Corke (2005).

2.10. Color measurement of noodle sheets

A Chroma meter (Minolta CR-100) equipped with D65 illuminant, using the CIE 1976 L^*, a^* and b^* color scale was used to measure the color of the fresh noodle sheets. L^* is a measurement of brightness (0–100), a^* represents the red–green coordinates (– is green while + is red) while b^* measures the blue–yellow coordinates (– is blue with + indicating yellowness) of a product. Samples were cut into pieces of about 5 cm in diameter and measured within 5 min. Each noodle group was measured for 6 times.

2.11. Statistical analysis

The data obtained in this study were expressed as the mean of at least three replicate determinations and standard deviation (SD) by using the software SPSS 16.0 for windows. Total plate count data in figures were transformed into logarithms of the number of colony forming units (CFU/g). Significance was defined at $P < 0.05$ by using Duncan's test.

3. Results and discussion

3.1. Preparation of MRPs

Absorbances at 280 nm and 420 nm are widely used as indicators of the yield of Maillard reaction. Absorbance at 280 nm is indicative of the formation of intermediate compounds of the Maillard reaction, while absorbance at 420 nm suggests the development of brown color. Fig. 1 shows the UV–vis absorbance changes of MRPs during heating. Xylose or chitosan showed no absorbance changes when heated alone, indicating that no disturbance was caused by caramelisation or Maillard reaction of chitosan itself. In contrast, a significant absorbance change was observed when mixtures were heated together. Both browning and UV absorbance showed a slight induction period at the beginning, and then came to a fast growth, suggesting a progressive accumulation of MRPs with the increase of time.

3.2. Antimicrobial activity of MRPs

The inhibition effect of chitosan/MRPs against test microorganisms was described in Table 1 by the method of minimum inhibitory concentration (MIC). With the extending of reaction time, the antimicrobial activity of chitosan against *Escherichia coli*, *Bacillus subtills*, *S. aureus* and *A. candidus* all increased at the early stage and then decreased or remained unchanged at the later stage. The MRPs of 6 h exhibited higher antimicrobial activity than others. Its MIC value against *A. candidus* decreased from 550 to 250 μg/ml. Results illustrated that Maillard reaction could enhance the antimicrobial activity of chitosan, the antimicrobial activity of MRPs did not necessarily attribute to the formation of melanoidins, and the brown color was not a good indicator for the antimicrobial activity of MRPs.

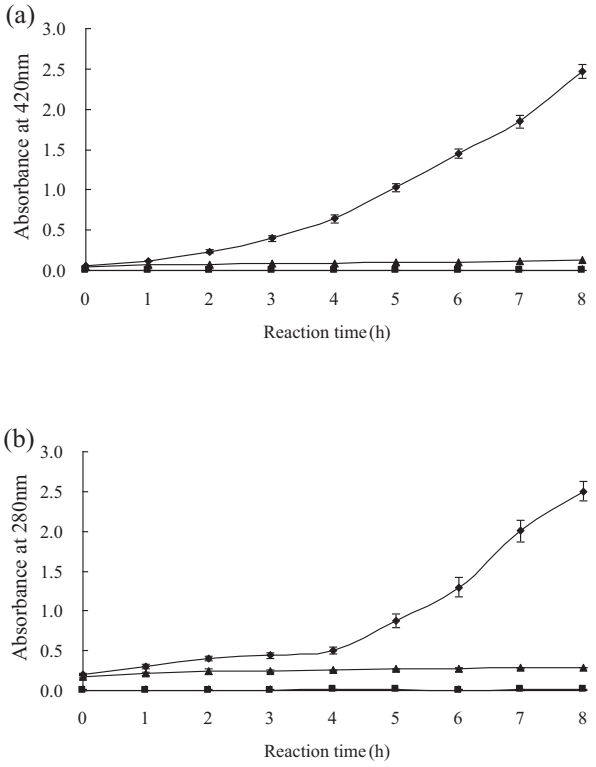

Fig. 1. Absorbance changes of MRPs prepared with chitosan and xylose over times at 80 °C: (a) measured at 420 nm, (b) measured at 280 nm (♦): chitosan–xylose mixtures; (■): xylose only; (▲): chitosan only.

This result was in accordance with the conclusion of Huang, Huang, Huang, and Chen (2007).

3.3. Antioxidant activity of the MRPs

The reducing power (a) and DPPH scavenging activity (b) of MRPs were presented in Fig. 2. The unheated samples showed only minor reducing power, while the heated samples rapidly attained fine reducing power depending on heating time. Different results have been reported previously upon the reducing power of chitosan MRPs. According to Kanatt et al. (2008), the chitosan–glucose Maillard reaction products did not show any noteworthy reducing power. But the result of Li, Shi, et al. (2011) showed that chitosan could attain significant reducing power through Maillard reaction, which was consistent with the result of this study. 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. In addition, as can be seen in Fig. 2b, MRPs were endowed with remarkable DPPH free radical scavenging activity. At the early stage, the prolonged reaction time leaded to rapidly increased scavenging activity. However, when the reaction time increased to 8 h, the scavenging activity of MRPs became slightly lower than that reacted for 6 h. The decrease in DPPH scavenging

Table 1
MIC of MRPs over different reaction time on main strains in semi-dried noodle (μg/ml).

Strains	Reaction time (h)				
	0	2	4	6	8
Escherichia coli	200	200	175	125	150
Bacillus subtills	250	225	200	150	200
Staphylococcus aureus	125	125	100	75	75
Aspergillus candidus	550	450	300	250	300

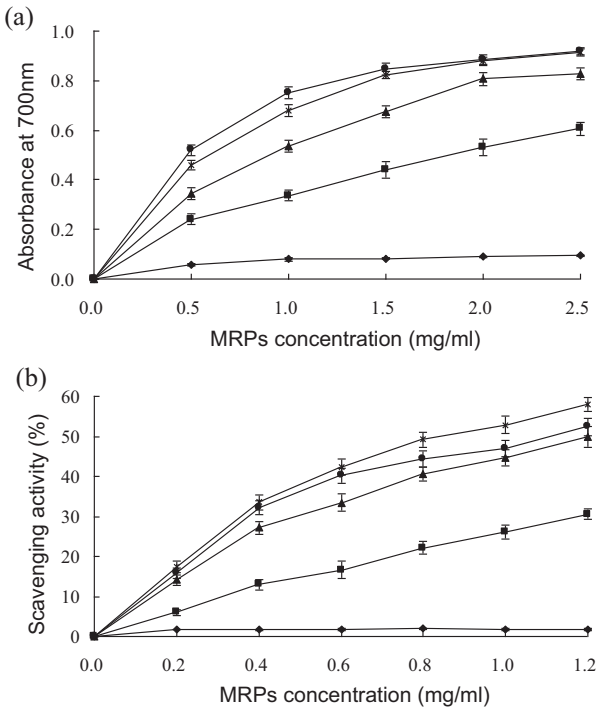

Fig. 2. Reducing power (a) and DPPH free radical scavenging effect; (b) changes of MRPs as reaction time increased. (♦) chitosan–xylose mixture without heating; (■) MRPs of 2 h; (▲) MRPs of 4 h; (*) MRPs of 6 h; (●) MRPs of 8 h.

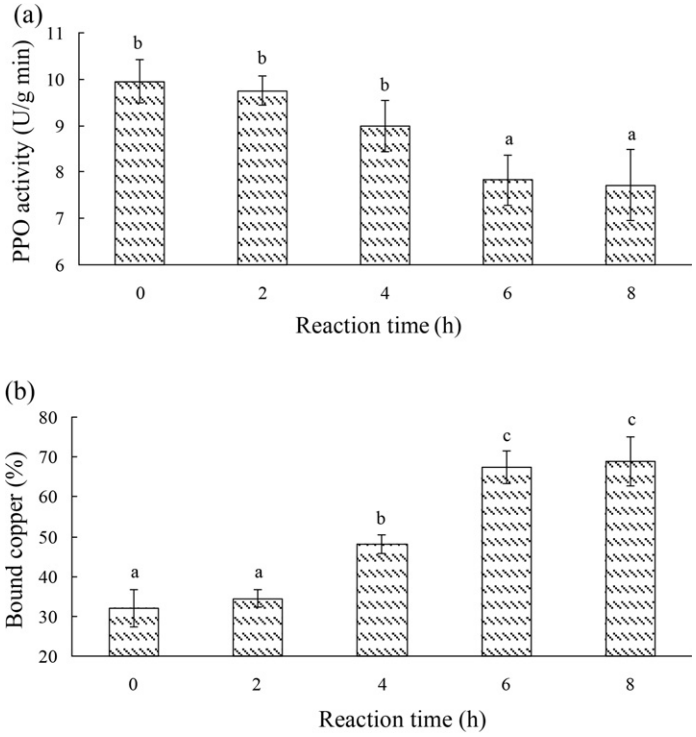

Fig. 3. PPO inhibitory effects (a) and copper-chelating activity (b) of MRPs over different reaction times.

activity may be due to the loss of some intermediate compounds, which possessed good antioxidant activity and were used to synthesize the final products of Maillard reaction.

3.4. PPO inhibitory effects and copper-chelating properties of MRPs

Color is considered as a major determinant of noodle quality (Li et al., 2012). One of the common faults of semi-dried noodles is that they are liable to darken, which would lead to the complicated marketability declines. To improve shelf-life it is also desirable to stop or at least slow the darkening (Asenstorfer, Appelbee, & Mares, 2009). Polyphenol oxidase (PPO), a type of copper containing oxidoreductase, was widely recognized as the predominant factor leading to the darkening of Chinese and Japanese noodles. Thus the inhibition techniques for PPO activity have drawn a considerable effort these years.

Inhibitory effects of MRPs synthesized for different times on PPO activity were exhibited in Fig. 3a. Compared to the unheated mixture, MRPs induced lower activity to the PPO extracts, and the inhibitory effects increased with reaction time. Some authors also reported that MRPs was the inhibitor of polyphenol oxidases in apple, banana and brinjal (Cheriot, Billaud, Pöchtrager, Wagner, & Nicolas, 2009; Lee, 2007; Tan & Harris, 1995). In fact, PPO from different food materials were quite different in their molecular structure, however, all of the PPO had an active center containing Cu^{2+} (Almeida & Nogueira, 1995). Fig. 3b shows the copper-chelating properties of MRPs heated for different times. The chitosan–xylose mixture without heating exhibited relatively low copper-chelating property while much higher chelating power was observed as the increase of reaction time. Therefore, efficiency of copper chelation could be improved by Maillard reaction. Some researchers reported that melanoidins of MRPs had the ability to form strong complexes with Cu^{2+} (Rendleman & Inglett, 1990). In addition, O'brien and Morrissey (1997) claimed that the Amadori rearrangement product, which was a key intermediate of early stage of Maillard reaction, had chelating properties as well. Thus the copper-chelating properties of MRPs may account for their inhibitory effect on PPO activity.

3.5. Effects of MRPs on the microbial growth in semi-dried noodles

To ascertain the antimicrobial and shelf-life extending properties of MRPs in semi-dried noodle system, products prepared by heating chitosan with xylose for 6 h were used to make noodles in an amount of 0.35%. Total plate count (TPC) in the control samples (noodles without any preservative) and noodles loaded with chitosan or MRPs were investigated respectively during storage. All the samples were placed under 25 °C and the results were presented in Fig. 4. The control noodle samples deteriorated quickly, whose TPC reached to 10^6 CFU/g (level of incipient spoilage (Li, Zhu, Guo, Peng, & Zhou, 2011)) after 6 days. The 0.35% chitosan enriched semi-dried noodles were more resistant to microbial growth than the control and their shelf-life was extended by 4 days.

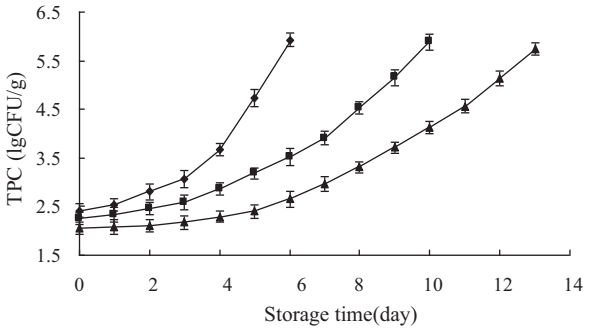

Fig. 4. Changes in viable bacteria count in semi-dried noodles as affected by MRPs at 25 °C. (♦) control; (■) semi-dried noodle with 0.35% chitosan; (▲) semi-dried noodle with 0.35% MRPs.

Table 2
Effect of MRPs on the initial color and color changes of semi-dried noodles.

Addition dose of MRPs (%)	L^*	a^*	b^*	ΔE_{ab}	$\Delta L^*_{0-24\,h}$
0	82.45 ± 0.17a	−4.54 ± 0.03a	19.52 ± 0.37a	–	2.13 ± 0.10a
0.15	82.29 ± 0.18ab	−4.48 ± 0.03a	20.22 ± 0.25b	0.7225	1.87 ± 0.07b
0.25	82.11 ± 0.06b	−4.34 ± 0.04b	20.98 ± 0.19c	1.5123	1.42 ± 0.09c
0.35	81.65 ± 0.16c	−3.98 ± 0.07c	21.52 ± 0.23c	2.2257	0.94 ± 0.08d

Values are shown as mean ± standard deviation. Means followed by the same letter in the same column are not significantly different ($P < 0.05$).

Table 3
Effect of MRPs on textural qualities and cooking loss of semi-dried noodles.

Addition dose of MRPs (%)	Hardness (g)	Springiness	Maximum shear force (g)	Breaking distance (mm)	Cooking loss (%)
0	5275.6 ± 150.3a	0.775 ± 0.010b	629.8 ± 18.1a	101.1 ± 6.7a	8.17 ± 0.07a
0.15	5476.8 ± 181.8a	0.810 ± 0.018a	645.5 ± 13.1a	103.5 ± 5.3ab	7.97 ± 0.04b
0.25	6106.3 ± 204.9b	0.818 ± 0.009a	674.6 ± 15.4b	108.9 ± 6.5ab	7.72 ± 0.07c
0.35	6549.1 ± 160.0c	0.828 ± 0.010a	681.9 ± 17.5b	112.7 ± 6.8b	7.72 ± 0.04c

Values are shown as mean ± standard deviation. Means followed by the same letter in the same column are not significantly different ($P < 0.05$).

Moreover, when 0.35% MRPs was added into semi-dried noodles, the induction period of the microbial growth curve became longer, and microbial growth rate was further slowed down. TPC in MRPs noodles reached to 10^6 after 13 days, indicating that MRPs of chitosan and xylose were more effective in prolonging the shelf life of semi-dried noodles.

3.6. Effects of MRPs on the color and darken rate of semi-dried noodles

Maillard reaction would impart a deep and brown color to chitosan, thus the color change in semi-dried noodles enriched by MRPs should be discussed. According to Asenstorfer, Appelbee, and Mares (2010), noodle sheet darkening can be measured by a change in the CieLab L^* value, which is a function of the diffuse light reflectance (scattering) as well as light absorption. Thus both physical changes and chemical changes can result in a change in the L^* value. The initial color and changes in L^* value of semi-dried noodles with addition of 0–0.35% 6 h MRPs were presented in Table 2.

As shown in Table 2, with the increase of MRPs, L^* value of the noodle sheet decreased while a^* and b^* value increased progressively, significant (although slight) differences were observed when the addition dose was 0.25% and 0.35%. The result indicated that adding MRPs into semi-dried noodle formulation may induce a darker and more yellow product. The concept of total color change (ΔE_{ab}), which is calculated the equation $\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$, was usually used to describe the visual sense differences among test samples. $\Delta E_{ab} \leq 1.5$ presents nearly no differences in visual inspection; $\Delta E_{ab} \geq 1.5$ is defined as being slightly different from the control; while $\Delta E_{ab} \geq 3.0$ indicates there is some differences between the samples. However, if ΔE_{ab} value exceeds 6.0, it means there is significant difference. Table 2 shows that noodle with 0.15% MRPs had little color change ($\Delta E_{ab} \leq 1.5$) visually, when the addition dose increased to 0.25–0.35%, the change in noodle color was still slight ($1.5 \leq \Delta E_{ab} \leq 3$). Thus, it could be concluded that despite the typical browning color MRPs itself, limited adding dose would not impart too much difference to the color of semi-dried noodle.

In addition, the darkening rate of semi-dried noodle with or without MRPs during 24 h storage time (expressed by $\Delta L^*_{0-24\,h}$) were determined and listed in Table 2. $\Delta L^*_{0-24\,h}$ of semi-dried noodles with 0.15% MRPs could be significantly ($P < 0.05$) reduced compared to the control and it further decreased as the addition dose increased to 0.25% and 0.35%. The result indicated that MRPs could effectively inhibit the darkening of semi-dried noodles. Their inhibitory effect on PPO activity as above mentioned might account for this phenomenon.

3.7. Effects of MRPs on the textural and cooking qualities of semi-dried noodles

Textural and cooking qualities are important concerns to consumers of noodle products. The instrumental parameters of hardness, maximum shear force, breaking distance and springiness are closely correlated with sensory texture characters of cooked noodles (Li et al., 2012). Cooking loss is referred as the total contents of solids present in gruel obtained from the cooked noodle. During cooking, soluble parts of noodle leach into the water and make the cooking water become cloudy and thick. This may be due to both amylose leaching and solubilization of some salt-soluble proteins (Petitot, Boyer, Minier, & Micard, 2010). Measuring cooking loss of these products is one of the important parameters in assessing their overall quality. Generally, noodle with appropriate high firmness, smoothness, springiness and low cooking loss is more desirable. Textural attributes and cooking loss of semi-dried noodles prepared with and without MRPs were measured and summarized in Table 3. It was shown that semi-dried noodle with 0.25–0.35% MRPs was significantly ($P < 0.05$) higher in hardness, springiness, maximum shear force, breaking distance and lower in cooking loss. This was probably attributed to the facts that MRPs possessed good surface activity (Brun-Mérimée, Billaud, Louarme, & Nicolas, 2004), which would interact with the gluten protein and starch molecules, helping to form a more compact gluten network.

4. Conclusions

It can be concluded that chitosan–xylose Maillard reaction products synthesized in this study were endowed with better antimicrobial and antioxidant activities than chitosan alone. Besides, the MRPs exhibited copper-chelating property and good inhibitory effect on PPO. Also, the addition of MRPs to semi-dried noodle could improve its textural and cooking qualities, slow down the darkening rate and impart a longer shelf-life to the products.

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