Optimized preparation, characterization, and antioxidant activity of chitooligosaccharide–glycine Maillard reaction products

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Revised: 29 November 2017 / Accepted: 4 December 2017 / Published online: 21 December 2017
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Abstract In this study, chitooligosaccharide (COS) and glycine (Gly) were selected to prepare Maillard reaction products, which were designated COS–Gly–MRPs. Changes in the FTIR and fluorescence spectra confirmed the formation of the COS–Gly–MRPs. Using ferric reducing antioxidant power (FRAP) as a response, the optimal reaction conditions, i.e., a time of 107 min, temperature of 121 °C, pH of 6.0, and $n_{\text{COS}}:n_{\text{Gly}} = 2.5:1$, were obtained by one-variable-at-a-time method and by response surface methodology. The resulting COS–Gly–MRPs exhibited much stronger antioxidant activity than their substrates. The FRAP of COS–Gly–MRPs was 32.14 mmol Fe²⁺/L, and the radical scavenging activity of COS–Gly–MRPs reached 78.6, 89.0, 92.3, and 86.0% for ABTS, superoxide, DPPH, and hydroxyl radicals, respectively. After 7 days of storage, COS–Gly–MRPs-treated fruit juices showed higher antioxidant capacity than those treated with a mixture of COS and Gly.

Keywords Antioxidant activity · Chitooligosaccharide · Glycine · Maillard reaction · Optimization

Introduction

The Maillard reaction, first discovered by Hellwig and Henle (2014), is a non-enzymatic browning reaction that involves a series of complex reactions between carbonyl-containing compounds and amino-containing compounds (Zhang et al. 2016). The Maillard reaction occurs naturally in food production or storage via the interaction of reducing sugars with amino acids and generates a wide range of Maillard reaction products (MRPs), resulting in the special odor, flavor, and color of food (Liu et al. 2012). MRPs possess numerous beneficial properties, such as antioxidant activity (Yin et al. 2014), antibacterial activity (Zhang et al. 2015), emulsification ability (Golkar et al. 2015), and the ability to improve the oxidative stability of food products (Wijewickreme et al. 1999). Of these various properties, the antioxidant activity of MRPs is of great interest for the enhancement of the antioxidant capacity of fruit juices. However, the antioxidant activity of MRPs varies with reaction substrate. Thus, it is necessary to evaluate the antioxidant activity and potential applications of MRPs derived from different reaction substrates.

Chitosan is the deacetylated derivative of chitin, the second most abundant biopolymer on the earth. Because of its favorable properties, e.g., antibacterial activity, antioxidant activity, biocompatibility, and biodegradability, chitosan is considered to be a novel biomaterial with multiple uses (Benhabiles et al. 2012). Chitosan possesses acetyl groups (CH₃CO-) at the C₂ position (Jung et al. 2014); thus, chitosan can be used to synthesize MRPs by reacting with amino-containing compounds. Furthermore, the desirable properties of chitosan are improved by the formation of MRPs. For example, the antioxidant property of chitosan is enhanced upon conjugation with β-lactoglobulin via the Maillard reaction (Mengibar et al. 2017). An

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13197-017-2982-0>) contains supplementary material, which is available to authorized users.

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improvement in antibacterial activity is observed for the ϵ -polylysine–chitosan conjugates produced by the Maillard reaction (Liang et al. 2014). However, the insolubility of chitosan in water limits the full exploitation of this biopolymer in practical applications. Fortunately, this shortcoming is easily overcome by the hydrolysis of chitosan to chitoooligosaccharide (COS). COS possesses better biological properties, including antioxidant activity, than chitosan. Consequently, COS has become a strong candidate for the preparation of MRPs, and MRPs with potent antioxidant activity have been synthesized successfully from COS alone (Jung et al. 2014). However, little information regarding MRPs derived from COS–amino acid model systems can be found in the literature.

Therefore, the aim of this study was to optimize the preparation of MRPs from COS and glycine (Gly), i.e., COS–Gly–MRPs, and assess their antioxidant activity. Moreover, the improvement in the antioxidant capacity of fruit juices was also evaluated after the addition of COS–Gly–MRPs.

Materials and methods

Materials

COS with a molecular mass of 3000 was purchased from Zhejiang Aoxing Biotechnology Co., Ltd., China. 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was obtained from Beijing Dingguo Changsheng Biotechnology Co., Ltd., China. 2,2'-Azinobis(3-ethylbenothiazoline-6-sulphonic acid) diammonium salt (ABTS) and 1,1-diphenyl-2-picryl-hydrazil (DPPH) were provided by Nanjing Oddfoni Biotechnology Co., Ltd., China. 3,5-Dinitrosalicylic acid was provided by Shanghai Kefeng Chemicals, China. 2,6-Dichlorophenolindophenol sodium was obtained from Shanghai Yuanye Biotechnology Co., Ltd., China. Other analytical-grade chemicals were obtained from Tianjin Yingdaxigui Chemicals, China.

Preparation and optimization of COS–Gly–MRPs

COS–Gly–MRPs were prepared as previously described (Luo et al. 2013) with some modifications. Typically, 2 g of COS was completely dissolved in 100 mL of distilled water, followed by the addition of Gly such that $n_{\text{COS}}:n_{\text{Gly}} = 1:1$ (mole ratio). The pH of the solution was adjusted to 5.0 with 1 mol/L acetic acid, followed by heating at 120 °C for 90 min. Then, the resulting COS–Gly–MRPs were cooled to 4 °C, characterized, and subjected to antioxidant capacity assays and application.

The reaction conditions, including time, temperature, pH, and $n_{\text{COS}}:n_{\text{Gly}}$, were determined by a one-variable-at-a-

time (OVAT) method and further optimized by response surface methodology (RSM).

In the OVAT experiments, the time was changed from 0 to 150 min, the temperature was set from 80 to 130 °C, the pH value was in the range of 3.0–6.0, and $n_{\text{COS}}:n_{\text{Gly}}$ was adjusted to 1:1, 1.5:1, 2:1, 1:1.5, and 1:2.

In this study, Box-Behnken Design (BBD), an RSM experimental design, was applied to assess the relationship between the antioxidant activity of COS–Gly–MRPs and the reaction conditions. BBD experiments were conducted using time (X_1), temperature (X_2), pH (X_3), and $n_{\text{COS}}:n_{\text{Gly}}$ (X_4) as independent variables and ferric reducing antioxidant power (FRAP) as a response. The values of each variable were based on the results of the OVAT experiments. A second-order polynomial regression model, as expressed in Eq. (1) (Zheng et al. 2014), was used to analyze the experimental data.

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k b_{ij} X_i X_j \quad (1)$$

where Y is the response; X_i and X_j are independent variables; b_0 is the constant coefficient; b_i , b_{ii} and b_{ij} are regression coefficients; and k is the number of independent variables.

Characterization of COS–Gly–MRPs

Fourier transform infrared (FTIR) spectroscopy of COS–Gly–MRPs, COS, and Gly was conducted on a KBr disc in a wavenumber range from 4000 to 400 cm^{-1} using a Vector22 FTIR spectrometer (Bruker, Germany).

The fluorescence spectra of COS–Gly–MRPs, COS, and Gly were determined using an F-4500 fluorescence spectrophotometer (Hitachi, Japan) as described by Luo et al. (2013). The excitation wavelength was 343 nm, and the emission wavelength ranged from 400 to 600 nm.

Antioxidant capacity assays

FRAP assay

The FRAP was determined as described by Mir et al. (2015) with modifications. The FRAP reagent, prepared by mixing 10 mmol/L TPTZ in 40 mmol/L HCl, 20 mmol/L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 0.3 mol/L acetate buffer (pH 3.6) in a ratio of 1:1:10, was incubated at 37 °C for 10 min. Then, 3 mL of the FRAP reagent was mixed with 0.5 mL of the sample solution, followed by incubation at 37 °C for another 10 min. Then, the absorbance of the mixture was measured at 593 nm using a UV-1100 ultraviolet–visible spectrophotometer (Mapada, China) and used to calculate the FRAP ($\text{mmol Fe}^{2+}/\text{L}$).

ABTS assay

The ABTS radical scavenging activity was assessed using a method described by Yu et al. (2013) with modifications. The ABTS solution was prepared by mixing an equal volume of a 7 mmol/L ABTS stock solution with a 2.45 mmol/L potassium persulfate solution. The mixture was then stored in the dark at room temperature for 12–16 h. The ABTS solution was diluted with 10 mmol/L phosphate-buffered saline (PBS, pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 50 μ L of the sample solution was added to 3 mL of the diluted ABTS solution. The absorbance of the mixture was immediately measured at 734 nm after a 6-min incubation in the dark at room temperature. The control was prepared by replacing the ABTS solution with PBS; the blank was prepared in an identical manner, substituting distilled water for the sample. The ABTS radical scavenging activity (%) was calculated as $[1 - (A_s - A_c)/A_0] \times 100$, where A_s , A_c , and A_0 are the absorbance values of the sample, control, and blank, respectively.

Superoxide radical assay

The superoxide radical scavenging activity was evaluated as described by a previous report (Li et al. 2009) with modifications. Typically, 1 mL of the sample solution was mixed with 3.2 mL of distilled water and 4.5 mL of Tris-HCl buffer (pH 8.2). The mixture was incubated at 25 °C for 20 min, followed by the addition of 0.3 mL of pyrogallol solution (60 mmol/L). The absorbance of the mixture was measured at 420 nm every 30 s for 5 min. The superoxide radical scavenging activity (%) was calculated as $(\Delta A_s - \Delta A_0)/\Delta A_s \times 100$, where ΔA_s and ΔA_0 refer to the changes in the absorbance values of the sample and the blank (in which the sample was replaced with distilled water), respectively.

DPPH assay

The DPPH radical scavenging activity was tested as described by Górnas et al. (2014) with modifications. Briefly, the DPPH solution (0.1 mmol/L) was freshly prepared by dissolving DPPH in ethanol and homogenizing in an ultrasonic bath for 30 s. Then, 2 mL of the sample solution was mixed vigorously with 2 mL of the DPPH solution, and the mixture was incubated in the dark at room temperature for 30 min. Then, the absorbance of the solution was measured at 517 nm. The DPPH radical scavenging activity (%) was calculated as $[1 - (A_s - A_c)/A_0] \times 100$, where A_s , A_c , and A_0 are the absorbance values of the sample, control (in which the DPPH solution was replaced with ethanol), and blank (in which the sample was replaced with ethanol), respectively.

Hydroxyl radical assay

The hydroxyl radical scavenging activity was determined using a method described by Heo et al. (2005) with modifications. A 1 mL aliquot of the sample solution was mixed with 1 mL of phenanthroline (1.5 mmol/L), 2 mL of PBS (20 mmol/L, pH 7.4), 1 mL of FeSO_4 solution (0.5 mmol/L), and 1 mL of H_2O_2 solution (0.1%, v/v). After incubation at 37 °C for 50 min, the absorbance of the mixture was measured at 536 nm. The hydroxyl radical scavenging activity (%) was calculated as $(A_s - A_0)/(A_c - A_0) \times 100$, where A_s , A_c , and A_0 are the absorbance values of the sample, control (in which the H_2O_2 solution was replaced with distilled water), and blank (in which the sample was replaced with distilled water), respectively.

Application of COS-Gly-MRPs in fruit juices

The antioxidant capacity of kiwifruit (*Actinidia chinensis*), pineapple (*Ananas comosus*), and peach (*Prunus persica*) juices treated with COS-Gly-MRPs was evaluated during storage by four assays, namely, the ascorbic acid (vitamin C) content assay, Folin-Ciocalteu reagent assay (FCR), DPPH assay, and FRAP assay. In brief, COS-Gly-MRPs were added into the fruit juices at concentrations of 1% (w/v), followed by storage at 4 °C. A 5 mL aliquot of fruit juice was withdrawn from each sample at different time intervals and evaluated for antioxidant capacity. The ascorbic acid content (g/L) of the fruit juices was determined by a titrimetric method using 2,6-dichlorophenolindophenol (AOAC 1995). Typically, 1 mL of fruit juice was mixed with 1 mL of 2% (w/v) oxalic acid solution, followed by dilution with 1% (w/v) oxalic acid solution to a volume of 10 mL. Then, the solution was titrated with a 0.02% (w/v) 2,6-dichlorophenolindophenol solution in 0.02% (w/v) sodium bicarbonate. The endpoint of the titration was reached when a faint pink color persisted throughout the solution for 15 s. The titrant was standardized by titration against 10 mL of a 0.1 g/L standard solution of ascorbic acid in 1% (w/v) oxalic acid solution. The FCR assay was conducted as described by Górnas et al. (2016) and the results were expressed as g of gallic acid per L of juice (g GA/L). In brief, 1 mL of fruit juice was added to 2.5 mL of Folin-Ciocalteu reagent. After a 5-min reaction, the solution was mixed with 2 mL of saturated Na_2CO_3 and diluted to a volume of 10 mL, followed by incubation at 30 °C for 2 h. Then, the absorbance of the solution was measured at 760 nm. For the DPPH and FRAP assays, the fruit juice was diluted 100-fold and 20-fold with distilled water, respectively; then, the diluted fruit juices were subjected to DPPH and FRAP assays, the procedures for which are described above. In addition, the fruit juices were also treated with a mixture of COS and

Gly (COS+Gly) before heating and evaluated in an identical manner.

Results and discussion

Preparation and optimization of COS–Gly–MRPs

FRAP reflects the reducing power of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) and is routinely used to analyze the total antioxidant activity of compounds. Herein, the FRAP of COS–Gly–MRPs obtained using different reaction conditions was analyzed by the OVAT method. First, the effect of time on the FRAP of COS–Gly–MRPs was studied; the results are presented in Fig. 1a. The FRAP of COS–Gly–MRPs increased sharply during the initial 30 min and gradually reached a maximum over 90 min. However, a slight decrease in the FRAP of COS–Gly–MRPs was found at time points beyond 90 min. MRPs consist of various antioxidant compounds, such as melanoidins, heterocyclic compounds, and reductones (Hellwig and Henle 2014). The levels of antioxidant compounds in the reaction increase over time, resulting in the enhancement of the FRAP. However, heating over a long period of time may cause the decomposition of COS–Gly–MRPs; hence, a decrease in the FRAP of COS–Gly–MRPs was observed at time points beyond 90 min. Moreover, the

FRAP observed for COS–Gly–MRPs was higher than that observed for COS or Gly alone, indicating the stronger antioxidant activity of COS–Gly–MRPs. Additionally, the Maillard reaction is highly sensitive to temperature. Usually, the reactivity between carbohydrates and amino acids has a positive correlation with the temperature (Langner and Rzeski 2013), and our results follow this trend. Upon raising the temperature from 80 to 130 °C, the FRAP of the COS–Gly–MRPs increased gradually (Fig. 1b). The pH value is another important factor affecting the preparation of MRPs. In this study, the effect of pH on the FRAP of COS–Gly–MRPs was evaluated from pH 3.0 to pH 6.0, and the results are shown in Fig. 1c. In general, higher pH values facilitate the formation of MRPs (Jiang et al. 2014). However, the solubility of COS decreased slightly with increasing pH values, which reduced the extent of completion of the Maillard reaction. Consequently, the maximum FRAP of COS–Gly–MRPs was observed at pH 5.5. The effect of $n_{\text{COS}}:n_{\text{Gly}}$ on the FRAP of COS–Gly–MRPs is shown in Fig. 1d. The FRAP of COS–Gly–MRPs increased significantly with increasing amounts of COS in the substrates, whereas increasing the amount of Gly did not lead to increased FRAP. This result reveals that COS is more strongly involved in the Maillard reaction than Gly, as also suggested by Martins and Van Boekel (2005). The melanoidin skeleton is mainly generated from the degradation of saccharides in the early stage of the Maillard reaction

Fig. 1 Effects of time (a), temperature (b), pH (c), and $n_{\text{COS}}:n_{\text{Gly}}$ (d) on the FRAP of COS–Gly–MRPs, COS, and Gly

(Yaylayan and Kaminsky 1998); thus, increasing the amount of COS facilitates the enhancement of the FRAP of COS–Gly–MRPs.

RSM is an attractive method to evaluate the relationship between a response and independent variables (Carciochi et al. 2016). Based on the results of the OVAT approach, the reaction conditions were further optimized by the BBD under RSM using the FRAP of COS–Gly–MRPs as a response. The BBD consisted of 29 experimental runs, including 5 central points, and the results are presented in Table 1.

To evaluate the significance of the model and the variables, an analysis of variance was performed for the second-order response surface model, and the results are presented in Table S1. A *P* value less than 0.05 indicates the statistical significance of the model terms (Zheng et al. 2014). In this case, first-order effects X_3 and X_4 , interaction effects X_1X_3 , X_1X_4 , and X_3X_4 , and second-order effects X_1^2

and X_3^2 were significant model terms. The *P*-value of lack-of-fit was greater than 0.05, suggesting that the model is significant and is a valid method for predicting the response. Thus, the model was used to determine the optimum combination of variables as well as the maximum FRAP.

The RSM optimization of the experimental data showed that the optimal reaction conditions were as follows: a time of 106.91 min, temperature of 121.04 °C, pH of 6.03, and $n_{\text{COS}}:n_{\text{Gly}} = 2.49:1$. In this case, the predicted FRAP was 32.17 mmol Fe²⁺/L. Considering the practicality of actual operation, the optimal conditions were adjusted to a time of 107 min, temperature of 121 °C, pH of 6.0, and $n_{\text{COS}}:n_{\text{Gly}} = 2.5:1$. Using the optimal conditions, verification experiments were conducted and repeated three times. Finally, a maximum FRAP of 32.14 ± 0.66 mmol Fe²⁺/L was obtained, which was very close to the predicted value. In contrast, the FRAP obtained using COS and Gly was

Table 1 BBD matrix and results

Run	Independent variables				FRAP (mmol Fe ²⁺ /L)	
	X_1 : Time (min)	X_2 : Temperature (°C)	X_3 : pH	X_4 : $n_{\text{COS}}:n_{\text{Gly}}$	Experimental	Predicted
1	90	130	4.5	1.5:1	22.79	23.31
2	90	130	6.5	2.5:1	30.76	30.35
3	120	130	5.5	2.5:1	30.70	30.10
4	120	130	6.5	2:1	24.89	25.01
5	90	120	5.5	2.5:1	30.51	30.79
6	120	130	5.5	1.5:1	22.36	21.85
7	120	140	5.5	2:1	24.20	25.10
8	90	130	5.5	2:1	29.16	29.46
9	90	120	6.5	2:1	26.45	26.97
10	90	140	5.5	2.5:1	31.24	30.85
11	90	130	6.5	1.5:1	23.35	23.05
12	60	130	5.5	2.5:1	25.52	26.24
13	60	120	5.5	2:1	25.91	25.10
14	90	120	4.5	2:1	23.82	23.65
15	60	130	4.5	2:1	22.79	22.37
16	90	130	5.5	2:1	29.32	29.46
17	90	130	5.5	2:1	29.46	29.46
18	90	130	5.5	2:1	29.96	29.46
19	60	140	5.5	2:1	26.37	26.37
20	90	130	5.5	2:1	29.38	29.46
21	60	130	5.5	1.5:1	24.72	25.53
22	90	140	6.5	2:1	25.75	26.12
23	60	130	6.5	2:1	23.08	22.78
24	90	140	4.5	2:1	24.63	24.32
25	120	120	5.5	2:1	26.45	26.55
26	120	130	4.5	2:1	20.32	20.31
27	90	130	4.5	2.5:1	24.57	24.97
28	90	120	5.5	1.5:1	26.38	26.46
29	90	140	5.5	1.5:1	26.80	26.22

only 2.733 ± 0.021 mmol Fe^{2+}/L and 0.174 ± 0.0038 mmol Fe^{2+}/L , respectively. These results confirmed, again, that COS–Gly–MRPs had much stronger antioxidant activity than COS and Gly.

Characterization of COS–Gly–MRPs

To identify COS–Gly–MRPs, the FTIR spectra of COS–Gly–MRPs, COS, and Gly were recorded; the results are shown in Fig. 2a. The FTIR spectrum for the original COS exhibited characteristic bands located at 1628 cm^{-1} (C=O stretching vibration of amide I), 1518 cm^{-1} (N–H bending vibration of amide II), 1321 cm^{-1} (C–N stretching vibration of amide III), 1155 cm^{-1} (asymmetric stretching vibration of C–O–C), 1072 cm^{-1} (C–O stretching vibration of $\text{C}_3\text{--OH}$), and 897 cm^{-1} (stretching vibration of saccharide ring) (Jing et al. 2016). Compared with COS, the band corresponding to amide II disappeared in the FTIR spectrum of COS–Gly–MRPs, indicating that no free amino groups exist in the product. Notably, a new band appeared in the COS–Gly–MRPs spectrum at 1734 cm^{-1} , which is assigned to the C=O stretching vibration of Amadori compounds formed in the early stage of the Maillard reaction (Horvat et al. 1997). These changes in the FTIR spectrum of COS–Gly–MRPs confirmed the occurrence of the Maillard reaction.

During the advanced stages of the Maillard reaction, some compounds with fluorescent properties are formed, and the fluorescence intensities of these compounds are used frequently as markers for the yield of MRPs (Luo et al. 2013). Gly did not exhibit this fluorescent property, whereas COS showed a fluorescence band centered at 432 nm (Fig. 2b). In contrast, COS–Gly–MRPs exhibited a fluorescence band at 449 nm, which was 17 nm redshifted with respect to the fluorescence band of COS, and the fluorescence intensity of COS–Gly–MRPs was much higher than that of COS. Similar properties were also observed in MRPs derived from other model systems. Jing and Kitts (2002) reported that the fluorescence intensities

of MRPs derived from glucose– and fructose–casein models increased gradually over time. Therefore, changes in the fluorescence spectrum provided evidence for the formation of COS–Gly–MRPs.

Free radical scavenging activity of COS–Gly–MRPs

In addition to the ability to act as a reducing agent, free radical scavenging activity is another important property of antioxidant compounds. In this study, COS–Gly–MRPs prepared under the optimal conditions were evaluated for free radical scavenging activity. The ABTS, superoxide, DPPH, and hydroxyl radical scavenging activity of COS–Gly–MRPs is shown in Fig. 3. ABTS radical scavenging activity is frequently used to measure the antioxidant properties of compounds. COS and Gly exhibited the ABTS radical scavenging activity of 54.4 and 17.4%, respectively. After the Maillard reaction, a sharp increase in the ABTS radical scavenging activity of COS–Gly–MRPs was observed. The ABTS radical scavenging activity of COS–Gly–MRPs reached 78.6%, which was 1.44- and 4.52-fold that of COS and Gly, respectively. Similar trends were also observed for the other three radicals. The scavenging activity of COS–Gly–MRPs for superoxide, DPPH, and hydroxyl radicals reached 89.0, 92.3, and 86.0%, respectively. The radical scavenging activity of MRPs derived from COS has also been previously studied. Jung et al. (2014) prepared MRPs from chitoooligomer and found that MRPs have good DPPH and ABTS radical scavenging activity. For the Maillard reaction, either the intermediates or the final products can act as hydrogen donors (Eric et al. 2013), which endows COS–Gly–MRPs with excellent radical scavenging activity.

Application of COS–Gly–MRPs in fruit juices

Because COS–Gly–MRPs exhibit potent antioxidant activity, these compounds might have potential application in fruit juices. Thus, the antioxidant capacity of kiwifruit,

Fig. 2 FTIR (a) and fluorescence (b) spectra of COS–Gly–MRPs, COS, and Gly

Fig. 3 Free radical scavenging activity of COS-Gly-MRPs, COS, and Gly

pineapple, and peach juices with the treatment of COS-Gly-MRPs and COS+Gly was measured during storage; the results are presented in Table 2. Ascorbic acid, a well-known antioxidant with health benefits (Bhat et al. 2013), makes a notable contribution to the antioxidant capacity of fruit juices (Gardner et al. 2000), and thus, ascorbic acid content in the three fruit juices was assessed during storage. Without any treatment, the ascorbic acid content of kiwi-fruit, pineapple, and peach juices decreased by 83.2, 76.8, and 72.0%, respectively, after 7 days of storage. However, upon treatment with COS+Gly, the ascorbic acid content decreased to 60.9–75.3%, indicating that the addition of COS+Gly to fruit juices slightly improved the ascorbic acid content. In contrast, after 7 days of storage, the ascorbic acid content of COS-Gly-MRPs-treated kiwifruit, pineapple, and peach juices decreased by 41.3, 34.0, and 43.8%, respectively, suggesting that COS-Gly-MRPs-treated fruit juices possessed a higher ascorbic acid content than the COS+Gly-treated juices. The FCR assay is a

Table 2 Antioxidant capacity of fruit juices treated with COS-Gly-MRPs and COS+Gly

Fruit juice	Treatment	Storage duration (days)	Ascorbic acid (g/L)	FCR (g GA/L)	DPPH scavenging activity (%)	FRAP (mmol Fe ²⁺ /L)
Kiwifruit juice	Control	0	3.16 ± 0.26	1.13 ± 0.03	64.63 ± 3.78	4.61 ± 0.27
		3	0.96 ± 0.08	1.04 ± 0.07	45.48 ± 1.09	2.43 ± 0.15
		7	0.53 ± 0.26	0.89 ± 0.04	22.71 ± 3.64	1.41 ± 0.18
	COS+Gly	0	3.20 ± 0.20	1.14 ± 0.03	67.49 ± 4.68	4.41 ± 0.20
		3	2.06 ± 0.20	1.00 ± 0.02	49.33 ± 4.31	2.94 ± 0.36
		7	0.79 ± 0.13	0.97 ± 0.02	32.33 ± 2.93	2.55 ± 0.13
	COS-Gly-MRPs	0	3.44 ± 0.03	1.13 ± 0.01	69.33 ± 2.05	4.63 ± 0.09
		3	3.25 ± 0.15	1.16 ± 0.01	57.53 ± 1.41	4.72 ± 0.11
		7	2.02 ± 0.15	1.09 ± 0.04	42.42 ± 3.15	4.14 ± 0.25
Pineapple juice	Control	0	1.51 ± 0.07	1.29 ± 0.01	62.08 ± 3.53	4.11 ± 0.10
		3	0.88 ± 0.08	1.09 ± 0.03	42.94 ± 1.95	2.22 ± 0.20
		7	0.35 ± 0.08	0.79 ± 0.02	20.29 ± 2.88	1.10 ± 0.08
	COS+Gly	0	1.54 ± 0.02	1.32 ± 0.02	65.25 ± 2.97	4.19 ± 0.21
		3	0.99 ± 0.05	1.11 ± 0.05	47.14 ± 2.43	2.19 ± 0.23
		7	0.55 ± 0.03	0.98 ± 0.03	29.44 ± 1.68	1.11 ± 0.15
	COS-Gly-MRPs	0	1.56 ± 0.04	1.29 ± 0.01	65.25 ± 1.65	4.17 ± 0.11
		3	1.33 ± 0.05	1.27 ± 0.02	53.69 ± 2.23	4.01 ± 0.19
		7	1.03 ± 0.03	1.24 ± 0.02	39.06 ± 2.45	3.69 ± 0.15
Peach juice	Control	0	0.50 ± 0.09	2.59 ± 0.09	63.16 ± 3.75	3.85 ± 0.17
		3	0.25 ± 0.02	1.52 ± 0.14	41.48 ± 2.74	2.22 ± 0.13
		7	0.14 ± 0.02	0.99 ± 0.12	19.88 ± 2.92	1.46 ± 0.10
	COS+Gly	0	0.46 ± 0.07	2.68 ± 0.12	64.01 ± 3.43	3.91 ± 0.23
		3	0.25 ± 0.02	1.86 ± 0.08	45.38 ± 2.06	2.53 ± 0.06
		7	0.18 ± 0.02	1.19 ± 0.11	29.56 ± 3.94	1.72 ± 0.09
	COS-Gly-MRPs	0	0.48 ± 0.08	2.68 ± 0.16	65.25 ± 2.12	3.90 ± 0.19
		3	0.35 ± 0.08	2.59 ± 0.12	53.58 ± 2.11	3.16 ± 0.13
		7	0.27 ± 0.02	2.18 ± 0.12	39.53 ± 2.40	2.54 ± 0.15

reliable method for measuring the reducing capacity of samples, as described by Górnaś et al. (2016); thus, the FCR assay was conducted in this study to assess the reducing capacity of the three fruit juices. After 7 days of storage, the FCR values of the COS–Gly–MRPs-treated fruit juices were markedly higher than those of the controls and the samples treated with COS+Gly, indicating that the fruit juices treated with COS–Gly–MRPs had higher reducing capacity. In addition, COS–Gly–MRPs showed a negligible FCR value of 0.00342 ± 0.00055 g GA/L. DPPH scavenging activity is commonly used to characterize the antioxidant capacity of biological materials (Górnaś et al. 2016). After 7 days of storage, addition of COS–Gly–MRPs to fruit juices showed higher DPPH scavenging activity than the COS+Gly-treated juices, indicating that the antioxidant capacity of fruit juices improved upon treatment with COS–Gly–MRPs. FRAP is an easy and accurate assay to measure the overall antioxidant capacity of fruit juices (Klimczak et al. 2007). Thus, the antioxidant capacity of the three fruit juices in this study was further evaluated by FRAP. As expected, the FRAP assay showed patterns similar to the ascorbic acid content, FCR, and DPPH scavenging activity assays. A large decrease in the FRAP values was seen for the controls and the COS+Gly-treated fruit juices, whereas a small decrease was observed for the COS–Gly–MRPs-treated fruit juices, indicating better antioxidant capacity of fruit juices treated with COS–Gly–MRPs. Therefore, it can be concluded that the addition of COS–Gly–MRPs to fruit juices increases the antioxidant capacity of these beverages.

Conclusion

In the present study, COS–Gly–MRPs were obtained by heating the mixture of COS and Gly in aqueous solution, and the reaction conditions were optimized by RSM. Under the optimal reaction conditions, i.e., a time of 107 min, temperature of 121 °C, pH of 6.0, and $n_{\text{COS}}:n_{\text{Gly}} = 2.5:1$, COS–Gly–MRPs with strong antioxidant activity were successfully prepared. The FRAP of COS–Gly–MRPs was 32.14 mmol Fe^{2+} /L, and the ABTS, superoxide, DPPH, and hydroxyl radical scavenging activity of COS–Gly–MRPs reached 78.6, 89.0, 92.3, and 86.0%, respectively. After 7 days of storage, COS–Gly–MRPs-treated fruit juices exhibited higher antioxidant capacity than fruit juices treated with a mixture of COS and Gly. Our results suggest that COS–Gly–MRPs derived from the COS–Gly model system have strong antioxidant activity, and the addition of COS–Gly–MRPs to fruit juices increases the antioxidant capacity of these beverages.

Acknowledgements This work was supported by the Natural Science Foundation of Hebei Province (No. B2016202111).

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