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Antibrowning activity of MRPs in enzyme and fresh-cut apple slice models

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Abstract

The present study examined various types of Maillard reaction products (MRPs) for their inhibitory effect on mushroom tyrosinase. Results showed that monosaccharide–GSH (glutathione) but not polysaccharide–GSH derived MRPs were more active than GSH in inhibiting mushroom tyrosinase. However, in fresh-cut apple slice model, surprisingly GSH performed much better than sucrose–GSH derived MRPs when the apple slices were stored at room temperature for 24 h. Further time-course study did find deterioration in tyrosinase mushroom inhibitory activity of sugar–GSH derived MRPs over time, suggesting the formed tyrosinase inhibitors in MRPs are unstable. Different combinations of chemical agents with sucrose–GSH derived MRPs were also investigated on apple slices. A synergistic effect was observed when sucrose–GSH derived MRPs (3.125 mM) were applied in combination with 0.5% ascorbic acid. Apart from instability of principal inhibitors, observation of an unpleasant odor from apple slices treated with MRPs raised another concern about the probable negative impact of these inhibitors on the sensory quality of food products. Our research indicates the limited application of MRPs as antibrowning agents for food products.

Keywords: Maillard reaction products; Tyrosinase inhibitors; Thiol compounds; Synergistic effect; Sensory quality

1. Introduction

In many edible plant products such as fresh fruits and vegetables and some seafoods (crustaceans), polyphenol oxidases (PPO) or tyrosinases catalyze the oxidation of phenolic constituents in the presence of oxygen. Majority of these enzymatic reactions have unfavorable effects on food quality, notably discoloration and darkening of food products. In addition, enzymatic browning is also deleterious to the nutritional value of foods. Therefore, for decades the food industry is constantly searching for effective and safe strategies to control such reactions. The demand for efficacious inhibitors of key enzyme tyrosinase has been increasing in recent years as people's living standards continue to improve with the accompanying greater demand for high quality health food products and convenient food products such as fresh-cuts fruits and vegetables.

Sulfites have long been used as a low cost additive in food products for their antibrowning and antimicrobial activity. However, their use in foods was banned by the USA Food and Drug Administration (FDA) in 1986 in view of their potential health risks to sensitive individuals (Buta, Moline, Spaulding, & Wang, 1999). In order to search for a safer and cost-effective substitute, chemical compounds and agents from natural sources have been explored for their antibrowning activity. Among the evaluated agents vitamin C, organic acids (oxalic acid, oxalacetic acid, citric acid), thiol-containing compounds (glutathione, cysteine, *N*-acetylcysteine) (Buta et al., 1999; Kwak & Lim, 2005; Son, Moon, & Lee, 2001), neutral sugars (glucose, fructose) (Osdzmianski & Lee, 1990), and natural extracts such as rhubarb juice and pineapple juice (Lozanodegonzalez, Barrett, Wrolstad, & Durst, 1993; Son, Moon, & Lee,

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2000), are potential inhibitors of enzymatic browning. The combinations of some of the above enzymatic inhibitors were also shown to possess remarkable antibrowning effects (Buta et al., 1999; Li, Cheng, Cho, He, & Wang, 2007; Nicoli, Anese, & Severini, 1994). Recently, Billaud et al. (2005b) have identified the Maillard reaction products (MRPs) as a novel class of potent antibrowning agents.

Maillard reaction is a kind of non-enzymatic browning reaction between reducing sugars and amino acids or proteins. Formation of MRPs is influenced by multiple factors such as reaction time, temperature, pH and solvents. Some MRPs, especially thiol-derived MRPs generated from heated mixtures of glucose or fructose and cysteine or GSH were shown to inhibit the activity of PPO using spectrophotometric and polarographic methods (Billaud, Brun-Merimee, Louarme, & Nicolas, 2005a; Billaud, Roux, Brun-Merimee, Maraschin, & Nicolas, 2003). Such potent inhibitory activity has been attributed to their antioxidant and copper chelating activities (Brun-Merimee, Billaud, Louarme, & Nicolas, 2004; Roux, Billaud, Maraschin, Brun-Merimee, & Nicolas, 2003). The structure–activity relationship analysis of MRPs has revealed that the concurrent presence of an amino group, a carboxyl group and a free thiol group in the same molecule are critical for strong inhibition of tyrosinase (Billaud, Maraschin, Peyrat-Maillard, & Nicolas, 2005c). Most of these previous studies on the identification of tyrosinase inhibitory MRPs were based on simple sugars. Little is known that whether the reaction products of polysaccharides and amino acids have tyrosinase inhibitory activity or not, while polysaccharides are important components of various food products, especially contributing to the textural properties in processed food products. In the present study, in concert with the effort to search for potent MRPs against enzymatic browning, various types of MRPs derived from simple sugar–GSH as well as polysaccharide–GSH models were investigated. Apart from enzyme-model assay, fresh-cut apple slice model was also employed to prove the value of potential inhibitors in practical applications. In light of our previous finding that oxyresveratrol in combination with ascorbic acid was the most effective in inhibiting enzymatic browning (Li et al., 2007), it is valuable to study whether the combination of oxyresveratrol and MRPs will show synergistic antibrowning effects since oxyreveratrol and MRPs are among the most promising antibrowning agents discovered recently.

2. Materials and methods

2.1. Materials

Reduced glutathione (GSH), (structure showed in Fig. 1) D-glucose, D-galactose, D-ribose, D-xylose, dextran, xanthan gum, guga gum, tragacanth gum, potato starch, ascorbic acid, L-tyrosine, mushroom tyrosinase (3900 units/mg) and silicone oil were purchased from Sigma–Aldrich Chemical Co. (St. Louis MO, USA). Sodium dihydrogen orthophosphate was obtained from BDH (Dorset, England). Sucrose, sodium hydrogen phosphate, pre-packed liChroprep RP-18 column (size B, 310–325, 40–63 μm) was purchased from Merck Co. (Darmstadt, Germany). HPLC grade solvents were purchased from BDH (Poole, UK). Oxyresveratrol (structure showed in Fig. 1) was isolated and purified according to the procedure in our previous study (Li et al., 2007). The Reacti-Therm III heating module (model 18840) and the screw cap Tuf-Bond Teflon fitted glass reaction vials (10 mL capacity) were purchased from Pierce Co. (Rockford, IL). Tyrosinase inhibitory activity was monitored on a UV-1206 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

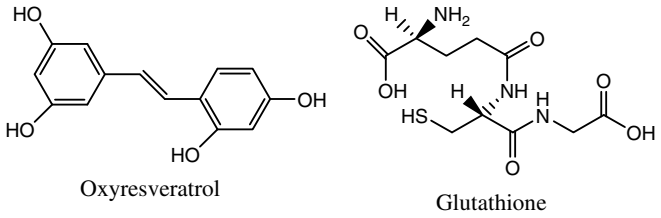

Fig. 1. Chemical structures of oxyresveratrol and glutathione.

2.2. Preparation of maillard reaction products (MRPs)

Same volume of solutions containing glutathione (0.25 M) and simple sugar (0.25 M) or polysaccharide (20 mg/mL) were heated in glass reaction vials to prepare different model aqueous MRPs. The vials were sealed with screw cap fitted with Tuf-Bond Teflon. 2 mL of silicone oil was added to each cavity of the heating block. The temperature meter of the heating block was then set to 103 °C and preheated for 2 h before inserting the vials into the cavities. The total heating time was 14 h (Billaud et al., 2005b) and fluctuation of temperature was within 1 °C. At the 14-h time point, the vials were immediately cooled in ice–water mixture. Aliquots from different reaction samples were diluted prior to determination of their antibrowning effect on tyrosinase and apple slices.

2.3. Mushroom tyrosinase inhibition assay

The assay was carried out according to the method of Vanni et al.(1990). L-Tyrosine was dissolved in 50 mM sodium phosphate buffer (pH 6.8) and used at a concentration of 0.1 mg/mL. Mushroom tyrosinase was also dissolved in the same buffer and used at a concentration of 200 units/mL. Different concentrations of GSH were first measured for their relative effectiveness (% inhibition) in inhibiting the activity of mushroom tyrosinase in order to determine its IC_{50} value (6.25 mM). Based on the concentration of GSH originally present in each model, dilution was made for the reaction mixtures such that their final concentration of GSH equals to the IC_{50} obtained above. Diluted sample solution was introduced into a cuvette, followed by addition of 1.0 mL L-tyrosine solution (0.1 mg/mL)

and finally 1.0 mL mushroom tyrosinase solution. The mixture was incubated at 37 °C for 20, 40 and 60 min and absorbance at 490 nm was measured. The absorbance of the same mixture with distilled water instead of sample solution was used as the control. All measurements were made in duplicate and mean values calculated. Percentage of inhibition of tyrosinase activity was calculated as follows:

$$\% \text{ inhibition} = [(C_t - C_0) - (T_t - T_0)]/(C_t - C_0) * 100,$$

where C_t = absorbance of blank solution after incubation, C_0 = absorbance of blank solution before incubation, T_t = absorbance of sample solution after incubation, and T_0 = absorbance of sample solution before incubation.

For testing the stability of MRPs, diluted samples (20×) from the ribose–GSH, xylose–GSH and sucrose–GSH models were allowed to stand under 23 °C for timed intervals (0, 2, 4, 8 and 20 h) and change in activity of these samples after incubation at 37 °C (for 40- and 60-min) was monitored.

2.4. Apple slice preparation and treatment

Fuji apples were bought from a local supermarket in Hong Kong. Apples were washed before cutting into 3-mm-thick slices. The apple slices were dipped in 300 mL of test solution for 3 min and drained. Control samples were dipped in distilled water. Samples were then placed in plastic Petri dishes with open access to air and stored at room temperature for 24 h. Hexplicate samples were prepared for each treatment, and the experiment was repeated twice. Test solutions used for the above samples included GSH (6.25 mM, 3.125 mM), MRPs (6.25 mM, 3.125 mM equivalent), oxyresveratrol (0.01%), GSH (6.25 mM, 3.125 mM) + oxyresveratrol (0.01%), MRPs (6.25 mM, 3.125 mM equivalent) + oxyresveratrol (0.01%), GSH (3.125 mM) + ascorbic acid (0.5%), MRPs (3.125 mM equivalent) + ascorbic acid (0.5%) and oxyresveratrol (0.01%) + ascorbic acid (0.5%).

2.5. Color measurements

Changes of color development in the samples were measured with a tristimulus reflectance colorimeter (Minolta CR-400 Chroma Meter). The center of the apple slices was in touch with the lens of the colorimeter when taking the readings (L^*, a^*, b^* values). Measurements were made immediately after the apple slices were treated and at time intervals thereafter. Total color difference (ΔE) was also used to evaluate the antibrowning potential of different treatments. ΔE was calculated as follows:

$$\Delta E = [(L_t^* - L_{\text{intial}}^*)^2 + (a_t^* - a_{\text{intial}}^*)^2 + (b_t^* - b_{\text{intial}}^*)^2]^{0.5}.$$

2.6. Separation of MRPs and HPLC analysis of fractions

The pre-packed ODS column (liChroprep RP-18 column, Merck, Darmstadt, Germany) was first eluted with acetonitrile (300 mL) to remove possible organic contaminants and then conditioned with water. 16 mL ribose–GSH derived MRPs was loaded onto the column using a PTFE tubing pump (Masterflex console drive, model 77390-00, Cole–Parmer Instrument Company). Elution was performed with various gradients of acetonitrile and water (0:100, 2:98, 5:95, 10:90, 20:80, 30:70, 40:60, 80:20), each of 200 mL and collected in 50-mL fractions to give a total of 32 fractions. Each fraction was concentrated to 5 mL under vacuum using a rotary evaporator. The concentrated samples were then compared for their activity in mushroom tyrosinase inhibition assay. Active fractions were analyzed by HPLC using a Shimadzu LC-20AT system equipped with a diode array detector (SPD-M20A) and a LC-Solution software (Shimadzu, Kyoto, Japan). Separation was performed on an Alltima C_{18} column (250 × 4.6 mm, 5 μm, Metachem Technologies Inc., Torance, CA, USA) and absorption spectra were recorded from 200 to 400 nm for all peaks. The mobile phase was composed of water with 0.1% formic acid (solvent A) and acetonitrile (solvent B). The flow rate was 1 mL/min. Two elution programs were applied. The first one was isocratic with 5% B and total run time was 25 min. The second one was a gradient program started with 5% B, which was held for 5 min. From 5 to 25 min, the gradient increased linearly to 30% B and kept at 30% B till 30 min, and then increased to 60% B from 30 to 40 min. The post-running time was 8 min.

2.7. Statistical analysis

The analysis of variance (AVOVA) and Tukey's multiple range test for comparison of means and the least significant differences were performed with the obtained data using the SAS system (SAS Institute, Inc, Cary, NC). $P < 0.05$ was selected as the lever decision for significant differences.

3. Results and discussion

3.1. Mushroom tyrosinase inhibitory activity of MRPs from different model reactions

The inhibitory activities of MRPs on mushroom tyrosinase have not been evaluated by any research group. In our current study, mushroom tyrosinase inhibitory activities of samples from different GSH-containing Maillard reaction models were compared with that of pure GSH using L-tyrosine as a substrate. As shown in Fig. 2, MRPs produced from simple sugars including glucose, ribose, xylose, galactose and sucrose in the presence of GSH were more active tyrosinase inhibitors than those produced from polysaccharide-containing models. Percentage of inhibition of sugar-derived MRPs was much higher than that of GSH alone at equivalent concentrations. On the other hand, polysaccharide-derived MRPs did not exhibit any significant difference in activity from GSH. It might be that either the thermal condition involved was not favorable for the

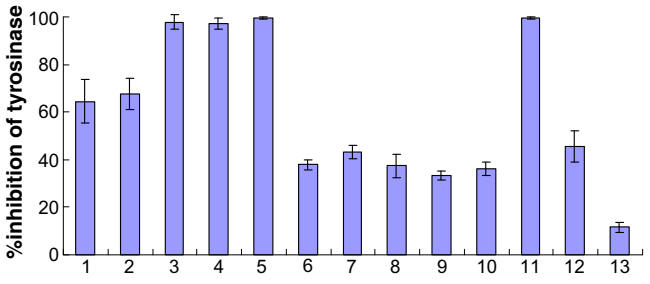

Fig. 2. Relative percentages of inhibition of tyrosinase of different Maillard reaction models and different concentrations of GSH. MRPs were prepared from GSH (0.25 M) and either sugars (0.25 M) or polysaccharides (20 mg/mL) at 103 °C for 14 h. Each MRPs was diluted 40 times. 1 – glucose + GSH, 2 – galactose + GSH, 3 – ribose + GSH, 4 – xylose + GSH, 5 – sucrose + GSH, 6 – dextran + GSH, 7 – xanthan gum + GSH, 8 – guar gum + GSH, 9 – tragacanth gum + GSH, 10 – potato starch + GSH, 11 – GSH (12.5 mM), 12 – GSH (6.25 mM), 13 – GSH (3.125 mM).

hydrolysis of these polysaccharides or many of their component monomers were inactive with respect to generation of potent tyrosinase inhibitory products in the presence of GSH. Among the simple sugars, ribose, xylose and sucrose showed comparable activity in terms of generation of tyrosinase inhibitory MRPs and so did glucose and galactose. However, the former three sugars were better than the latter two. In addition, activity of ribose-, xylose- and sucrose-derived MRPs was higher than twice that of GSH alone at equivalent concentration (6.25 mM). The finding for the better activity of pentose than that of hexose was in agreement with previous reports (Billaud et al., 2005b,c) although these studies used apple PPO instead. Billaud et al. (2005b) reported that in the presence of cysteine, pentoses were more reactive than sucrose in the generation of PPO inhibitors. Interestingly, with the use of GSH, pentoses and sucrose showed comparable activity in the present study, probably due to the different reaction mechanisms between cysteine and GSH with sucrose or its hydrolysis products.

3.2. Antibrowning activity of MRPs and GSH in apple slice model

To confirm the potential of tyrosinase inhibitory MRPs in practical applications, fresh-cut apple slices were chosen as our food model. In recent years, parameters such as ΔE, h^*, C^*, L^*, a^* and b^* were used frequently for measuring the extent of browning reaction in fruits and vegetables. Usually, a decrease in L^* means an increase in darkness, while an increase in a^* means an increase in redness. Earlier research studies have found that a^* value was more sensitive to browning in fresh-cut pear (Abbott & Buta, 2002; Sapers & Miller, 1998) and apple (Rojas-Grau, Sobrino-Lopez, Tapia, & Martin-Belloso, 2006). The a^* value was thus chosen as the main marker parameter for evaluating the antibrowning activity of the proposed inhibitors in this study. Since our enzyme-model investigation found MRPs from sucrose–GSH model was the most active one, it was selected as the first one for food application study. Moreover, in order to optimize the activity of such MRPs, testing candidates also included oxyresveratrol and ascorbic acid, which in our previous study (Li et al., 2007), were found to be an effective formulation against enzymatic browning in apple juice and slice models, most probably via a synergistic mechanism. In this regard, various combinations of purported tyrosinase inhibitors were examined.

The first set of tests (Table 1) started with a lower concentration of inhibitors (3.125 mM MRP or GSH) with/without 0.01% oxyresveratrol and 0.5% ascorbic acid. Among the different treatments, 0.01% oxyresveratrol + 0.5% ascorbic acid represented the best antibrowning formula during the 24 h testing period based on the change of a^* value and visual observation. This provided strong support for the prominent role of oxyresvertrol as an antibrowning agent. In addition, browning in apple slices was significantly inhibited ($P < 0.05$) by the three treatments containing GSH (GSH alone, GSH + ascorbic acid, GSH + oxyresveratrol) over the 24-h testing period. However, no significant difference ($P < 0.05$) in effectiveness was found among the three treatments themselves. In other words, neither ascorbic acid nor oxyresveratrol was able to achieve a synergistic effect with GSH under this set of experimental parameters. For sucrose–GSH derived MRPs, combination with 0.5% ascorbic acid or 0.01% oxyresveratrol both showed significant inhibition of browning ($P < 0.05$) with better activity based on the change of a^* value than that achieved with the MRPs alone after 6 h. However, these combinations showed worse activities than those containing GSH at the same concentration after 24-h

Table 1
a^* value of apple slices treated with tyrosinase inhibitory agents[a]

Tested solution	a^* value at designated time interval after treatment		
	6 h	12 h	24 h
Control	−1.73 ± 0.46 a	−1.66 ± 0.45 a	−1.66 ± 0.49 b
3.125 mM sucrose–MRPs	−2.86 ± 0.62 b	−1.74 ± 0.75 a	−0.37 ± 0.58 a
3.125 mM sucrose–MRPs + 0.01% oxyresveratrol	−3.41 ± 0.56 c	−2.98 ± 0.60 b	−1.48 ± 0.98 b
3.125 mM sucrose–MRPs + 0.5% ascorbic acid	−3.59 ± 0.56 c	−3.03 ± 0.79 b	−2.26 ± 0.95 c
3.125 mM GSH	−3.59 ± 0.37 c	−3.45 ± 0.33 b	−3.00 ± 0.42 d
3.125 mM GSH + 0.01% oxyresveratrol	−3.47 ± 0.51 c	−3.29 ± 0.51 b	−2.86 ± 0.56 d
3.125 mM GSH + 0.5% ascorbic acid	−3.58 ± 0.43 c	−3.37 ± 0.42 b	−3.12 ± 0.36 d
0.01% oxyresveratrol + 0.5% ascorbic acid	−4.11 ± 0.23 d	−4.01 ± 0.24 c	−3.94 ± 0.23 e

[a] Each value is expressed as the mean ± standard deviation ($n = 10$). Means with different letters in the same column are significantly different ($p < 0.05$).

treatment based on change of a^* value and visual observation. After 24-h, a^* value of samples treated with 3.125 mM MRPs + 0.01% oxyresveratrol rose to a level close to the control, while for treatment with MRPs alone, a^* value of the samples even increased to beyond that of the control after 24 h. The results are different from what we expected at the beginning. These observations suggest that GSH had stronger antibrowning effect than MRPs produced from the reaction of GSH and sucrose although in the initial mushroom tyrosinase inhibitory assay, the MRPs showed much stronger activity.

We thus carried out further tests to evaluate how MRPs generated from sucrose and GSH would behave at higher concentration. MRPs at 6.25 mM GSH equivalent, a concentration equal to that used in the tyrosinase inhibition assay, was chosen. As shown in Fig. 3, similar results were observed based on the change of a^* and total color difference (ΔE). GSH alone and GSH + 0.01% oxyresveratrol were capable of significantly inhibiting browning reactions in fresh-cut apple slices over the 24-h period, though the later treatment did not demonstrate significantly better activity than the former (statistical data not shown). In contrast, MPRs (6.25 mM) alone was only effective within 12 h of application. After that, a^* value of samples subjected to its treatment rose rapidly to a level much higher than the control at the 24-h time point, similar to its behavior at lower concentration (3.125 mM) in the first set of tests. In combination with 0.01% oxyresveratrol, improvement in activity was observed. Moreover, this combination demonstrated a synergistic effect for the first 12 h of application. After 12 h, a^* value of the samples treated with 6.25 mM MRPs + 0.01% oxyresveratrol increased at a high rate to approaching that of samples treated with 0.01% oxyresveratrol alone. This set of tests again suggested GSH showed stronger and more long-lasting antibrowning effects than MRPs generated from the reaction of sucrose and GSH. Similar results are also observed for MRPs generated from the reaction of GSH with other mono-sugars.

It is well established that Maillard reaction consists of a complex network of addition/condensation, rearrangement and degradation reactions and the reaction products may be unstable. The deterioration in antibrowning activities of MRPs could be caused by their degradation. The following stage of the study aimed to gain insight into the stability of the purported tyrosinase inhibitory MRPs.

3.3. Stability of diluted MRPs monitored by tyrosinase inhibition assay

To find out whether the anti-tyrosinase components of GSH-containing Maillard reaction models are stable, a time-course study was conducted for the three most active MRP mixtures derived from ribose–, xylose– and sucrose–GSH, respectively. Results are presented in Table 2. Deterioration in inhibitory activity to various extents was observed within the time program of this experiment. Based on the spectrophotometric data collected for the 40 and 60-min incubation time points (37 °C), over 50% reduction in mushroom tyrosinase inhibitory activity was found for MRPs from all three models after 20-h storage under room conditions, suggesting the corresponding tyrosinase inhibitory compounds are unstable, thus limiting their application in food science. However it is not known whether stability and thus tyrosinase inhibitory activity of the above MRPs could be substantially extended if cooler storage conditions such as 4–10 °C were applied.

3.4. Tyrosinase inhibitory activity and HPLC chromatogram of MRPs fractions

With the aim of acquiring an insight into the chemistry of MRPs capable of suppressing tyrosinase activity, we first

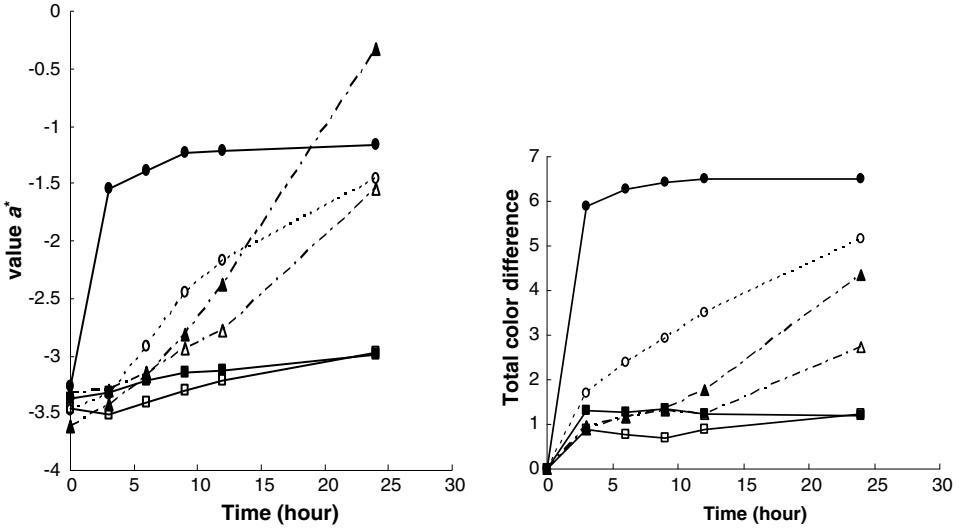

Fig. 3. Reflectance measurement of a^* and total colour difference (ΔE) of apple slices treated with the following tested solutions: (—●—) control (distilled water), (··○··) 0.01% oxyresveratrol, (—■—) 6.25 mM GSH, (—□—) 6.25 mM GSH + 0.01% oxyresveratrol, (—▲—) 6.25 mM sucrose–MRPs, (-·-△-) 6.25 mM sucrose–MRPs + 0.01% oxyresveratrol.

Table 2
Tyrosinase inhibitory activity of diluted ribose–, xylose–, and sucrose–GSH derived MRPs and GSH (6.25 mM) solution as a function of time and incubation temperature

Maillard reaction model	Time interval after dilution (hour)	% inhibition at timed intervals[a]	
		40 min	60 min
Ribose–GSH	0	94.57 ± 4.95	60.20 ± 5.58
	2	85.84 ± 7.16	54.54 ± 4.42
	4	70.27 ± 3.42	47.23 ± 3.15
	8	50.40 ± 8.43	36.85 ± 6.60
	20	34.30 ± 5.26	26.09 ± 3.63
Xylose–GSH	0	82.55 ± 20.22	50.49 ± 13.78
	2	44.64 ± 8.86	29.03 ± 6.20
	4	34.38 ± 6.82	21.22 ± 7.84
	8	29.74 ± 4.06	17.91 ± 3.56
	20	22.54 ± 3.77	12.04 ± 3.05
Sucrose–GSH	0	93.03 ± 8.26	49.14 ± 8.85
	2	83.50 ± 11.50	42.31 ± 3.67
	4	62.75 ± 9.53	34.89 ± 4.43
	8	53.97 ± 7.31	32.47 ± 5.05
	20	40.29 ± 2.88	25.19 ± 1.90
GSH		28.08 ± 2.09	11.35 ± 2.48

[a] Each value is expressed as the mean ± standard deviation ($n = 6$). GSH ($n = 15$).

chose the ribose–GSH model for activity-guided chromatographic analysis. Thirty-two fractions were collected from ODS column chromatography of ribose–GSH derived MRPs. These fractions were compared for their tyrosinase inhibitory activity to locate the active candidates. Fig. 4 presents their relative percentages of inhibition on tyrosinase activity. It is clearly shown multiple components contribute to the tyrosinase inhibitory effects of MRPs. Moreover, some of the fractions including Fr. 2, 4, 5, 10–12 and 22 were capable of achieving over 90% inhibition in this parameter. These seven fractions were thus subjected to HPLC analysis for comparison of their constitutional profiles. Fractions with similar profiles (Fr. 4–5 and Fr. 10–12, respectively) were combined to give a single pooled fraction. Fr. 2 and Fr. 4–5 contained only few dominant components. Nonetheless, several attempts with ordinary column chromatography failed to isolate the corresponding principal component due to its exceptionally high polarity. The remaining promising fractions were all of multi-component nature. Preparative HPLC of Fr. 10–12 afforded two major components. However, the two purified components turned out to be inactive in the subsequent tyrosinase inhibition assay. Therefore, we proposed that multi-components in the MRPs prepared from robose–GSH model system are likely to contribute to its inhibitory effect observed in tyrosinase inhibition assay. Some of these compounds may be UV-inactive while some of them may be prone to degradation/modification to inactive compounds during chromatographic processes. MRPs produced from the other sugar–GSH models were believed to have similar properties. Therefore, further attempts were not made to separate the corresponding active components.

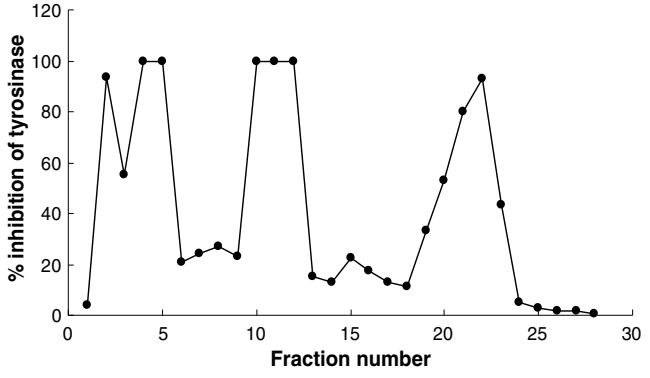

Fig. 4. Inhibitory activity of different fractions obtained from ODS column chromatography of ribose–GSH derived MRPs. Each value is expressed as the mean value of three replications, fractions after No. 28 have low inhibitory activity and data are not shown here.

In summary, the present study found that monosaccharide–GSH but not polysaccharide–GSH derived MRPs were more active than GSH in inhibiting mushroom tyrosinase. However, in fresh-cut apple slice model, GSH performed better than sucrose–GSH derived MRPs. A synergistic effect was observed when sucrose–GSH derived MRPs (3.125 mM) were applied in combination with 0.5% ascorbic acid. Synergism with 0.01% oxyresveratrol was only significant within 12 h of application. The decreased antibrowning activity of MRPs derived from GSH and sucrose may be due to instability of the active components. Attempt to purify the tyrosinase inhibitors from monosaccharide–GSH derived MRPs found multiple components contributed to its activity. Purification of these compounds is difficult to be managed with ordinary column chromatography. GSH–sugar and cysteine–sugar derived MRPs have recently been hot topics in the area related to the development of antibrowning agents. However based on our observation, the stability issue really will limit their

application in food. In addition, during the period of observation, we found that apple slices treated with MRPs had an unpleasant odor which was particularly obvious after long-term storage. Further studies with the participation of well trained sensory panel members are required to confirm the value of the above MRPs in practical applications.

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