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Oxyresveratrol as an Antibrowning Agent for Cloudy Apple Juices and Fresh-Cut Apples

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Antibrowning activities of *Morus alba* L. twig extracts, oxyresveratrol, and mulberroside A isolated from mulberry twig on cloudy apple juices and fresh-cut apple slices were evaluated by monitoring the change of *a** value, total color difference (ΔE), and visual observation. It was found, similar to 4-hexylresorcinol, that oxyresveratrol could effectively inhibit browning in cloudy apple juices at a concentration as low as 0.01% and that mulberry twig extract also showed remarkable antibrowning effects on cloudy apple juices. However, for fresh-cut apples slices, mulberry twig extract and oxyresveratrol needed to be used in combination at least with ascorbic acid to exhibit their antibrowning effects. Apple slice samples treated by dipping in a solution containing 0.001 M oxyresveratrol, 0.5 M isoascorbic acid, 0.05 M calcium chloride, and 0.025 M acetylcysteine did not undergo any substantial browning reaction for 28 days at 4 °C. However mulberroside A did not show antibrowning effects on cloudy apple juices although it is also a good mushroom tyrosinase inhibitor.

KEYWORDS: Antibrowning agent; oxyresveratrol; *Morus alba* L. extract

INTRODUCTION

During the past few years there has been a large increase in the demand for fresh-cut fruits and fruit juices, which attract customers as natural, fresh, minimally processed fruit products. However, as enzymatic browning (catalyzed by polyphenol oxidases (PPO)) becomes significant upon exposure of these products to air, the fruit processing industry is constantly confronted with the challenge of maintaining the appearance of these products. Apart from browning catalyzed by PPO, nonenzymatic browning reaction may also degrade organoleptic properties of the fruit products. Therefore, development of effective strategies to inhibit browning has been a critical part in the production process. Both physical and chemical approaches have been extensively tested and applied. Traditionally, low-temperature storage and modified atmosphere packaging are preferred physical methods to control browning. Recently, several new processing technologies, such as thermal treatment (*1*), application of edible coatings (*2*), high-pressure treatment (*3*), and treatment with supercritical carbon dioxide (*4*), have been proposed to prevent browning in fresh fruit products. For the chemical approach, the most important and probably most effective method is by addition of PPO inhibitors to suppress enzymatic browning and reductants to counteract nonenzymatic browning (*5*).

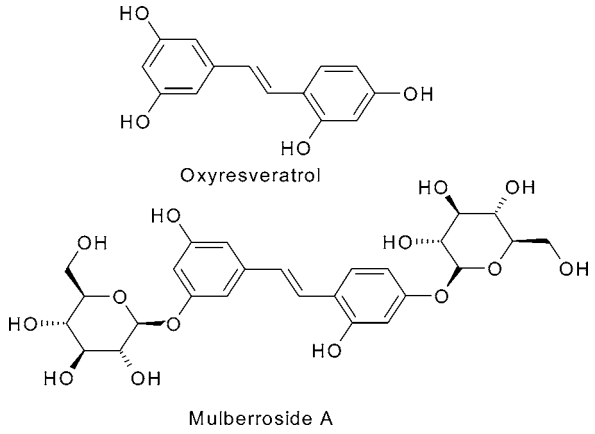

Figure 1. Chemical structures of oxyresveratrol and mulberroside A.

Sulfiting agents have been well-known for their antibrowning and antimicrobial effects on fresh-cut fruits and vegetables; however, their use on fruits and vegetables was banned in 1986 by the FDA owing to their potential hazards to health (*6*). Considerable work has been conducted to look for their substitutes, mainly natural alternatives. In recent years, a number of natural candidates were found to demonstrate satisfactory inhibitory activity. Examples are ascorbic acid and its derivatives, thiol-containing amino acids (*7*−*9*), kojic acid (*10*), citric acid, oxalic acid (*11*), 4-hexylresorcinol (*12*), honey (*13*),

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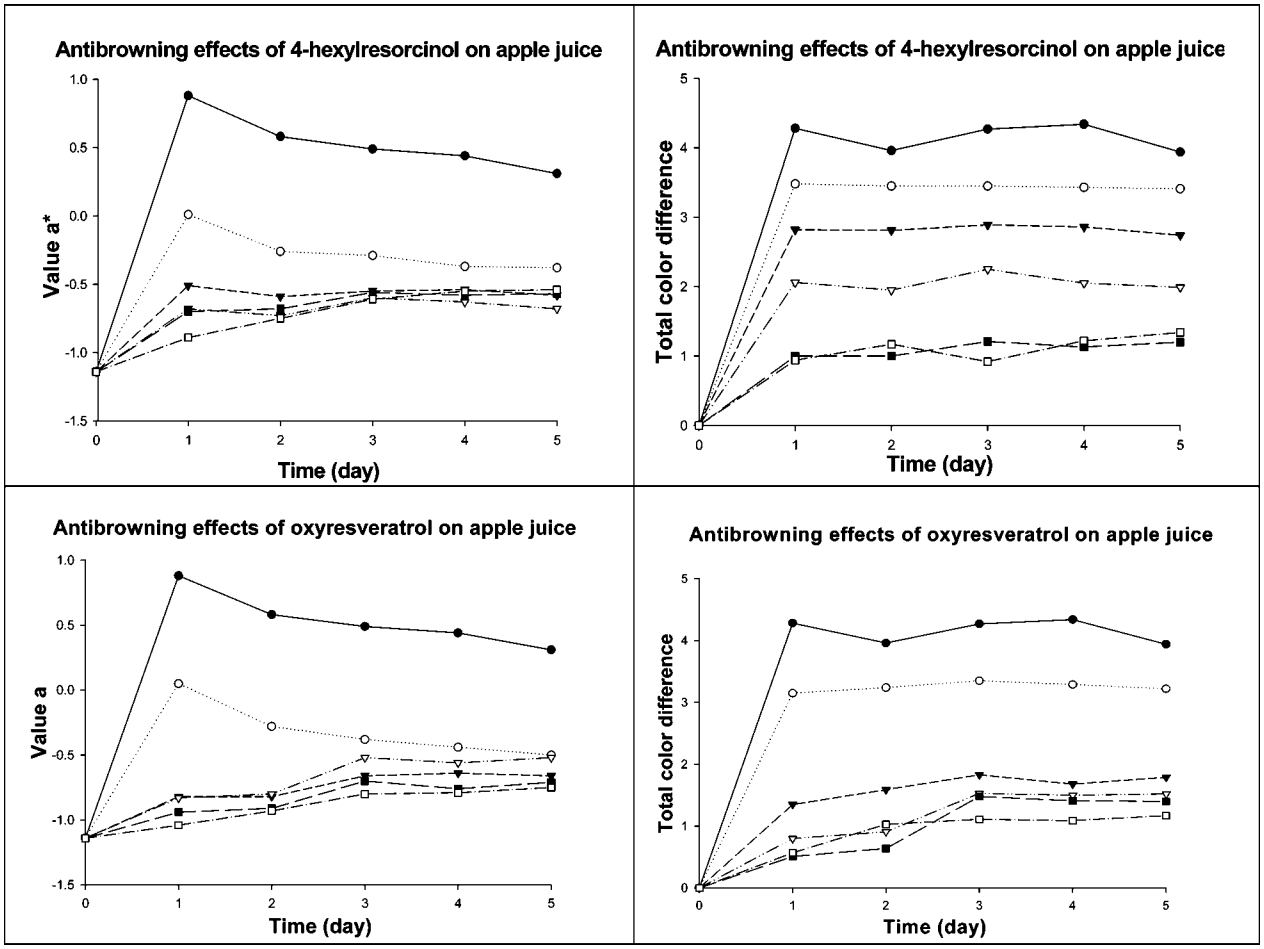

Figure 2. Reflectance measurement of *a** and total color difference (ΔE) of apple juices treated with 4-hexylresorcinol or oxyresveratrol in concentrations ranging from 0.0005% to 0.01% with 0.02% ascorbic acid and stored at 4 °C: -–●– control, --○-- 0.0005%, -–▼– 0.001%, –▽-– 0.002%, –■– 0.005%, –□– 0.01%.

pineapple juice, rhubarb juice, and licorice extract (*14*). So far, ascorbic acid and its derivatives are the most potential candidates to substitute sulfites. However, their antibrowning activity is temporary, and accelerated browning in certain products may take place when their reducing capacity is depleted (*15*). For thiol-containing organic compounds, applicability is limited by the consideration that their addition at high levels may give an unpleasant odor to fruit products. Further limitation arises from their weak penetrating ability which largely compromises their effectiveness in deep tissues (*16*). 4-Hexylresorcinol has been well known for its effectiveness in suppressing browning in shrimps (*17*). Its applicability in fresh fruits and vegetables has also been proven in several apple cultivars, pears, and radishes, especially when used in combination with reducing agents (*18*, *19*). However, it is still sub judice regarding the safety of its use.

The genus *Morus* is of great economical and medical importance. The mulberry leaves are the foods for silk worms, and the leaves, root barks, and twigs have all been used in oriental medicines to treat diabetes, arthritis, rheumatism, and aching and numbness of the joints (*20*, *21*). The mulberry fruit is edible and has been used to brew wine in China. In our previous study, *Morus alba* L. young twig extract exhibited potent inhibitory effects on mushroom, murine, and human tyrosinase and melanin synthesis in B-16 melanoma cells (*22*). Bioassay-guided separation revealed oxyresveratrol and mulberroside A as the principle ingredients, and it was found that oxyresveratrol potently inhibited mushroom tyrosinase with an IC_{50} value of 2.4 μM, which is 15-fold more potent than kojic acid (*22*). Moreover, oxyresveratrol demonstrated much stronger antioxidant activity than resveratrol (*23*), a well-known antioxidant produced by members of the *Vitaceae* in response to infection or injury. On the basis of the findings that oxyresveratrol demonstrated dual activities both as a tyrosinase inhibitor and as a strong antioxidant, it was hypothesized as a promising antibrowning agent for fruit and vegetable products. Nevertheless, to the best of our knowledge, neither oxyresveratrol, mulberroside A, nor *Morus alba* L. extract has been investigated for their efficacy in suppressing browning in foods. Therefore, the aim of this study is to examine their potential for practical application as antibrowning agents, especially for cloudy apple juices and fresh-cut apple slices.

MATERIALS AND METHODS

Plant Materials. Dried twigs and fruits of *Morus alba* L. were purchased from a local oriental herb vendor in Hong Kong. Fuji apple cultivar was chosen for investigation because of its popularity among different apple cultivars available on the market. The apples were obtained from a local supermarket and stored at 4 °C until used for analysis.

Chemicals and General Procedures. 4-Hexylresorcinol, ascorbic acid, isoascorbic acid, *N*-acetylcysteine and calcium chloride were purchased from Sigma (St Louis, MO). Sephadex LH-20 was purchased from GE Healthcare Bio-Sciences AB (Uppsala Sweden). Silica gel (130−270 mesh), TLC plates, and all organic solvents were obtained from Merck (Darmstadt, Germany). ^{1}H NMR was obtained on an INOVA-400 instrument, and LC-MS was run on an Agilent LC-MSD system equipped with an electrospray ionization source, Bruker

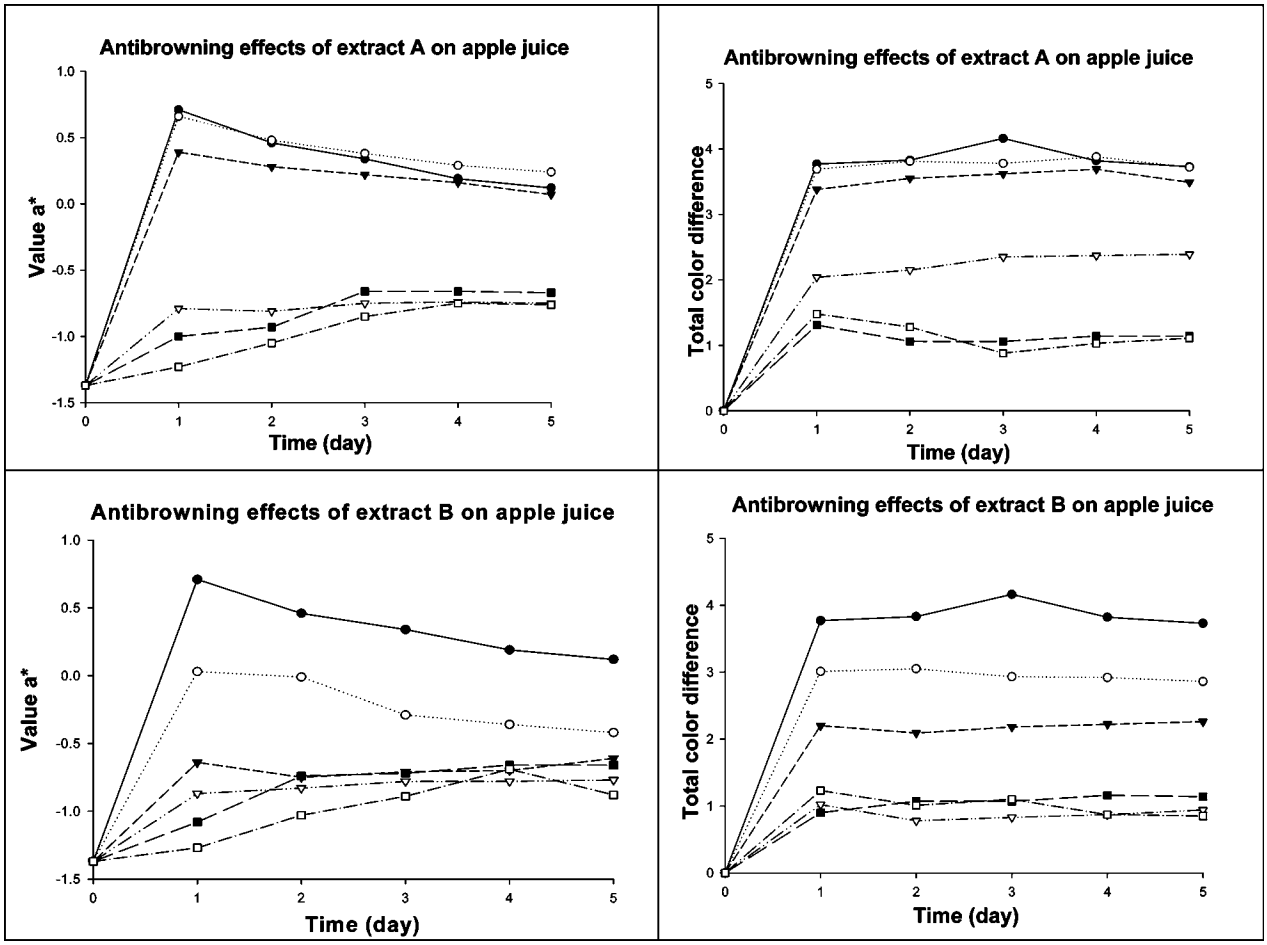

Figure 3. Reflectance measurement of a^* and total color difference (ΔE) of apple juices treated with mulberry twig extracts in concentrations ranging from 0.001% to 0.02% with 0.02% ascorbic acid and stored at 4 °C: -–●– control, --○-- 0.001%, -–▼– 0.002%, –▽-– 0.005%, –■– 0.01%, –□– 0.02%.

Table 1. a^* Value of Cloudy Apple Juices Treated with Different Concentrations of 4-Hexylresorcinol and Oxyresveratrol Solutions with 0.02% Ascorbic Acid[a]

	a^* value at designated time interval after treatment	
antibrowning agent	1 day	5 days
control (water)	0.88 ± 0.03 a	0.31 ± 0.12 a
0.0005% 4-hexylresorcinol	0.01 ± 0.10 b	–0.38 ± 0.14 b
0.0005% oxyresveratrol	0.05 ± 0.07 b	–0.50 ± 0.03 c
0.001% 4-hexylresorcinol	–0.51 ± 0.07 c	–0.58 ± 0.06 cde
0.001% oxyresveratrol	–0.82 ± 0.02 e	–0.66 ± 0.16 def
0.002% 4-hexylresorcinol	–0.68 ± 0.06 d	–0.68 ± 0.04 ef
0.002% oxyresveratrol	–0.83 ± 0.04 ef	–0.52 ± 0.10 c
0.005% 4-hexylresorcinol	–0.70 ± 0.03 d	–0.57 ± 0.07 cd
0.005% oxyresveratrol	–0.94 ± 0.05 g	–0.71 ± 0.09 f
0.01% 4-hexylresorcinol	–0.89 ± 0.06 fg	–0.54 ± 0.05 c
0.01% oxyresveratrol	–1.04 ± 0.02 h	–0.75 ± 0.08 f

[a] Each value is expressed as the mean ± standard deviation ($n = 6$). Means with different letters in the same column are significantly different ($p < 0.05$).

Table 2. a^* Value of Cloudy Apple Juices Treated with Different Concentrations of Mulberry Twig Extract A and Extract B Solutions with 0.02% Ascorbic Acid[a]

	a^* value at designated time interval after treatment	
antibrowning agent	1 day	5 days
control (water)	0.71 ± 0.01 a	0.12 ± 0.11 ab
0.001% extract A	0.66 ± 0.05 a	0.24 ± 0.05 a
0.001% extract B	0.03 ± 0.09 c	–0.42 ± 0.06 c
0.002% extract A	0.39 ± 0.09 b	0.07 ± 0.04 b
0.002% extract B	–0.64 ± 0.06 d	–0.61 ± 0.05 d
0.005% extract A	–0.79 ± 0.05 e	–0.75 ± 0.03 e
0.005% extract B	–0.87 ± 0.02 f	–0.77 ± 0.03 ef
0.01% extract A	–1.00 ± 0.04 g	–0.67 ± 0.28 de
0.01% extract B	–1.08 ± 0.05 h	–0.66 ± 0.01 ed
0.02% extract A	–1.23 ± 0.05 i	–0.76 ± 0.14 ef
0.02% extract B	–1.27 ± 0.07 i	–0.88 ± 0.03 f

[a] Each value is expressed as the mean ± standard deviation ($n = 6$). Means with different letters in the same column are significantly different ($p < 0.05$).

Daltonics 4.0, and Data analysis 4.0 software. A Waters Delta 600 liquid chromatograph system equipped with a 2487 dual-wavelength detector, a Masslynx V4.0 software, and a YMC ODS-AQ column (250 × 4.6 mm, 5 μm) was used for analytical purpose.

Preparation of *Morus alba* L. Twigs Extracts. In order to get detailed and complete information about antibrowning activity of *Morus alba* L. twig extract, twigs with different physiological ages were used to prepare extracts, with extract A coming from young twigs of *Morus alba* L., and extract B from older ones. Briefly, the dried twigs of *Morus alba* L. (200 g each) were ground to powders and sonicated in methanol (600 mL × 3) at room temperature for 2 h, then the plant materials were filtered off, and the extracts were combined and concentrated under reduced pressure using a rotor evaporator. Finally, 9.4 g of extract A and 7.8 g of extract B were obtained respectively and stored at 4 °C before use.

Isolation of Oxyresveratrol and Mulberroside A. The obtained dry extract (7 g) from old twig of *Morus alba* L. was dissolved in 100 mL of deionized water, and the aqueous solution was partitioned with chloroform, ethyl acetate, and *n*-butanol successively. The ethyl acetate fraction (3.5 g) was further purified by gel filtration on an Sephadex

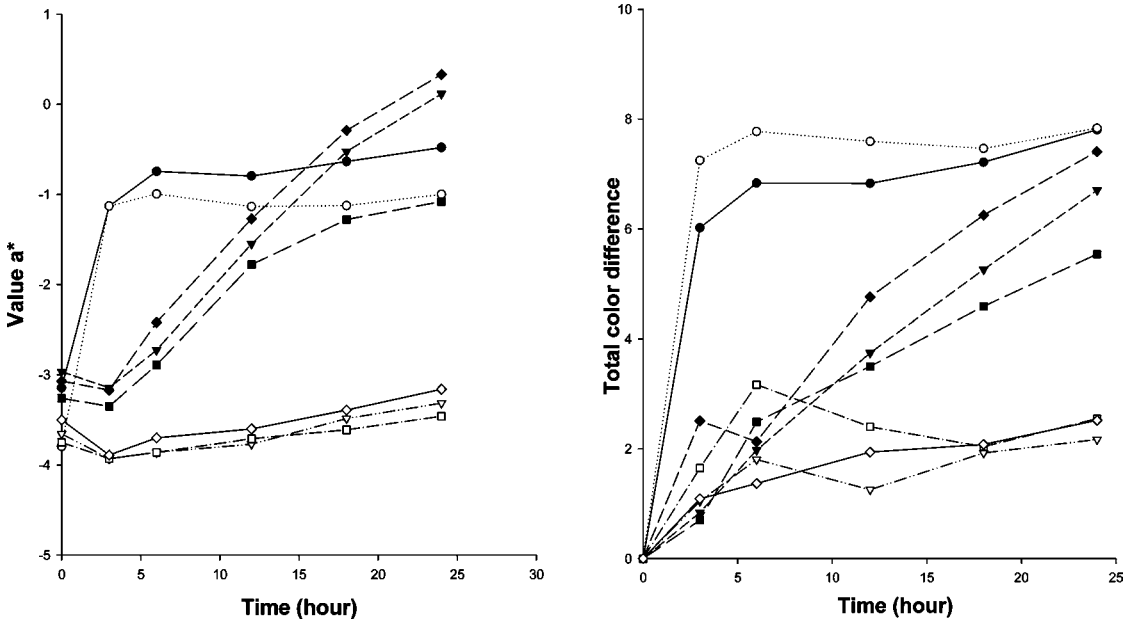

Figure 4. Reflectance measurement of *a** and total color difference (ΔE) of apple slices treated with the following tested solutions and stored at room temperature: - –●– control (distilled water), --○-- 0.5% ascorbic acid, - –▼– 0.01% 4-hexylresorcinol, –▽- – 0.01% 4-hexylresorcinol + 0.5% ascorbic acid, –■– 0.01% oxyresveratrol, –□– 0.01% oxyresveratrol + 0.5% ascorbic acid, –◆– 0.1% crude extract B, –◇– 0.1% crude extract B + 0.5% ascorbic acid.

LH-20 column (eluted with methanol) and adsorption chromatography on a silica gel column, eluted with a solvent system of chloroform/methanol/water (6:1:0.1, v/v/v) for isolation of oxyresveratrol. Mulberroside A was isolated from the *n*-butanol fraction as described in our previous study (*22*). The structures of oxyresveratrol and mulberroside A (**Figure 1**) were confirmed by MS and NMR spectral data.

Spectrometric Identification of Oxyresveratrol and Mulberroside A. Oxyresveratrol (2,3′,4,5′-tetrahydroxystilbene): Positive ESI-MS *m/z* 245.2 $[M + 1]^+$; ^{1}H NMR (400 MHz, in CD_3OD) δ 7.22 (1H, d, J = 8.8 Hz), 7.16 (1H, d, J = 16.4 Hz), 6.70 (1H, d, J = 16.4 Hz), 6.33 (2H, d, J = 2.4 Hz), 6.20 (2H, m), 6.03 (1H, t, J = 2.4 Hz). Mulberroside A: Positive ESI-MS *m/z* 569.4 $[M + 1]^+$; ^{1}H NMR (400 MHz, in CD_3OD) δ 7.33 (1H, d, J = 8.8 Hz), 7.21 (1H, d, J = 16.4 Hz), 6.84 (1H, d, J = 16.4 Hz), 6.66 (1H, s), 6.50 (3H, m), 6.35 (1H, d, J = 2.0 Hz), 4.79 (1H, d, J = 6.8 Hz), 4.76 (1H, d, J = 7.3 Hz), 3.20−3.85 (m).

HPLC Analysis of Oxyresveratrol in Mulberry Fruits and Twigs. Mulberry fruit (about 1 g), young twig (about 250 mg), and old twig (about 250 mg) ground powders were accurately weighed into 50 mL volumetric flasks. Then 40 mL of 70% methanol was added to each flask, followed by sonication at room temperature for 1 h. The flasks holding the extracts were cooled to room temperature and filled to volume using 70% methanol. After centrifugation at 14000 g for 5 min, the supernatants were collected for HPLC analysis. Oxyresveratrol standard (5 mg) was prepared in 25 mL of 70% methanol. An YMC ODS-AQ column (250 × 4.6 mm, 5 μm) was selected for HPLC analysis with a flow rate of 1.0 mL/min at ambient temperature. The mobile phase was composed of water (with 0.5% formic acid v/v, solvent A) and acetonitrile (solvent B) in a gradient system: initially 10% B, linear gradient to 50% B in 30 min, then linear gradient to 90% B in 3 min. The total running time was 33 min, and postrunning time was 15 min. Detection was conducted at 310 nm and quantification was based on the integrated peak areas with reference to an external standard. The naturally occurring amounts of oxyresveratrol in mulberry fruits and young twigs were found to be at 0.01% and 0.13%, respectively.

Apple Juice Preparation and Treatment. Two different methods, one without initial addition of ascorbic acid and the other with known concentration of ascorbic acid, were used for preparation of the apple juice samples. For the first method, apples were peeled, cored, sliced, and then homogenized in a homogenizer for 1 min with an equal amount of distilled water. Accurately weighed amounts of tested chemicals and extracts were directly added into the homogenizer before commencement of homogenization. The homogenate was pressed through a piece of cheesecloth to remove large particles. All samples were stored at room temperature for 24 h.

With the second method, ascorbic acid was added at the time of juicing to give a final concentration of 0.02% ascorbic acid. Tested compounds and extracts of different concentrations were then added after the bulk quantity of juice has been prepared and measured into corresponding test tubes. Control juice samples were treated with distilled water. All treated samples and control in this group were stored at 4 °C for 5 days.

Apple Slice Preparation and Treatment. Apples of comparable size were cleaned and cut into 4 mm-thick slices. All samples were treated by dipping the apple slices into 100 mL of test solution for 3 min and drained. Control samples were dipped in distilled water. Samples were then placed in plastic Petri dishes, sealed with Parafilm, and stored either at room temperature for 24 h or at 4 °C for 28 days. Hexplicate samples were prepared for each test, and the experiment was repeated three times.

Test solutions used for the above samples included ascorbic acid (0.5%), 4-hexylresorcinol (from 0.01% to 0.2%), oxyresveratrol (from 0.01% to 0.2%), mulberroside A (from 0.02% to 0.2%), mulberry twig extract A (0.1%), mulberry twig extract B (0.1%), ascorbic acid (0.5%) + 4-hexylresorcinol (0.01%), ascorbic acid (0.5%) + oxyresveratrol (0.01%), ascorbic acid (0.5%) + mulberry twig extract A (0.1%), ascorbic acid (0.5%) + mulberry twig extract B (0.1%), 0.001 M 4-hexyylresorcinol + 0.5 M isoascorbic acid + 0.05 M calcium chloride + 0.025 M acetylcysteine, and 0.001 M oxyresveratrol + 0.5 M isoascorbic acid + 0.05 M calcium chloride + 0.025 M acetylcysteine.

Color Measurements. Visual assessment of color development in the samples was performed with a digital camera while the relative extents of browning were measured with a tristimulus reflectance colorimeter (Minolta CR-400 Chroma Meter). For juices, 30 mL of thoroughly mixed juice was poured into the liquid tester of Minolta CR-400 Chroma Meter for measurement of L^*, a^*, and b^* values. For apple slices, the center of the apple slices was in touch with the lens of the Minolta CR-400 Chroma meter when taking the readings (L^*, a^*, and b^* values). Measurements were made immediately after the tested materials were treated and at timed intervals thereafter. Total color difference (ΔE) was also used to evaluate the antibrowning potential of different treatments. ΔE was calculated as follows: $\Delta E = [(L^*_t - L^*_{initial})^2 + (a^*_t - a^*_{initial})^2 + (b^*_t - b^*_{initial})^2]^{0.5}$.

Statistical Analysis. The analysis of variance (ANOVA) and Tukey's multiple range test for comparison of means and 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 level decision for significant differences.

RESULTS AND DISCUSSION

Although there has not yet been a unified standard quantitative method to monitor browning reaction in foods, for measurement of the extent of browning in fruit and vegetable products, the most frequently applied parameters are ΔE, h^*, L^*, a^*, and b^*. Usually, a decrease in L^* and an increase in a^* or ΔE mean the occurrence of browning. Earlier research studies have found that a^* value was more sensitive to browning and has been used to effectively monitor browning in fresh-cut pear (*24*, *25*) and apple (*18*). A recent research study found that a^* and ΔE (total color difference) were the best parameters to monitor browning on fresh-cut Fuji apple surfaces (*9*). This agrees with our preliminary observation, and a^* and ΔE were thus chosen as marker parameters for estimating the antibrowning activity of the proposed inhibitors in the present study. Using 4-hexylresorcinol, isoascorbic acid, and/or ascorbic acid as reference compounds, two potent mushroom tyrosinase inhibitors, oxyresveratrol and mulberroside A, together with one mulberry young twig extract (extract A) and one mulberry old twig extract (extract B), were evaluated for their antibrowning effects on cloudy apple juices and fresh-cut apple slices. Comparison was made via visual observation and based on changes of a^* value (measured by a chroma meter) and the total color difference (ΔE). Various combinations of oxyresveratrol or mulberroside A or mulberry twig extract with other antibrowning agents were also examined to see if any synergistic effect could be achieved and thus enables full utilization of their antibrowning capacities.

Antibrowning Activity of Mulberry Twig Extracts and Oxyresveratrol in Apple Juice Model. The antibrowning activities of oxyresveratrol, mulberroside A, and mulberry twig extracts were first evaluated by their direct addition into fresh-prepared cloudy apple juice samples. It was found that 0.01% oxyresveratrol and 0.1% mulberry twig extract showed significant antibrowning effect in the 24 h observation period for juice stored at room temperature, similar to that achieved with 0.01% 4-hexylresorcinol, and better than that with 0.01% isoascorbic acid. Surprisingly, mulberroside A, another major component of *Morus alba* L. young twigs which was proved to be an excellent melanogenesis inhibitor with low cytotoxicity in our previous work (*22*), did not show antibrowning activity; instead its treatment actually promoted browning when compared with the control. In this group of testing we also found that the antibrowning effect of isoascorbic acid (at 0.01% level) on apple juice was temporary and lasted for less than 1 h. Moreover, based on the pictures taken at the 24 h time-point, enhanced browning was observed, similar to the reports for ascorbic acid (*9*, *18*). It is well-known that ascorbic acid and its analogues may sometimes promote discoloration as such group of antibrowning agents are also antioxidants which may act as prooxidants, especially at low concentrations. However, such antioxidant/prooxidant theory could not explain the similar phenomenon observed in apple samples treated with mulberroside A. The contradictory results obtained in the test using mushroom tyrosinase and that using apple juice may probably arise from the different PPO profiles and contents in mushroom and apple (*26*).

In another set of apple juice samples, 0.02% ascorbic acid (final concentration) was added at the time of juicing in order to achieve a more unified initial color index, which formed the reference point for latter evaluation of the differential progression of browning in the control and in samples intervened with the tested chemicals or extracts. On the basis of the changes of a^* value, total color difference (ΔE) (**Figures 2** and **3**), and visual observation, oxyresveratrol, 4-hexylresorcinol and the two mulberry twig extracts demonstrated a dose-dependent antibrowning activity when combined with ascorbic acid.

Table 3. a^* Value of Apple Slices Treated with Different Antibrowning Agents in Water[a]

antibrowning agent	a^* value at designated time interval after treatment	
	3 h	24 h
control (water)	–1.13 ± 0.96 a	–0.48 ± 1.26 ab
0.5% ascorbic acid	–1.13 ± 1.28 a	–1.00 ± 1.14 b
0.01% 4-hexylresorcinol	–3.15 ± 0.58 b	0.12 ± 1.40 a
0.01% oxyresveratrol	–3.35 ± 0.30 bc	1.13 ± 2.18 b
0.1% extract B	–3.17 ± 0.47 b	0.33 ± 0.96 a
0.01% 4-hexylresorcinol + 0.5% ascorbic acid	–3.92 ± 0.41 d	–3.31 ± 0.57 c
0.01% oxyresveratrol + 0.5% ascorbic acid	–3.93 ± 0.35 d	–3.46 ± 0.47 c
0.1% extract B + 0.5% ascorbic acid	–3.89 ± 0.35 cd	–3.16 ± 0.41 c

[a] Each value is expressed as the mean ± standard deviation ($n = 18$). Means with different letters in the same column are significantly different ($p < 0.05$).

On the basis of the change of a^* value, oxyresveratrol showed better activity than 4-hexylresorcinol with significant statistical difference ($p < 0.05$) at two effective concentrations 0.005% and 0.01% during the 5 days' testing period (**Table 1**). For the two extracts, extract B was much more potent in suppressing apple juice browning based on visual observation and total color difference (ΔE); however based on the change of a^* value, at the two effective concentrations 0.01% and 0.02%, no statistical differences ($p < 0.05$) were observed between these two extracts after 5 days of treatment (**Table 2**). The striking difference between the two extracts may be related to their different contents of oxyresveratrol as supported by subsequent quantitative HPLC analysis, which revealed that the oxyresveratrol content of extract A and B was 1.7% and 5.4%, respectively. However, it should be noted that the two extracts at 0.01% in apple juice (equivalent to 0.00017% oxyresveratrol for extract A and 0.00054% for extract B in apple juice) showed much more powerful antibrowning activity than oxyresveratrol at the concentration of 0.0005% (**Figures 2** and **3**). This phenomenon indicates the existence of some modifying effects on the final antibrowning activity by other components in these extracts. They could be PPO inhibitors and/or reductants, etc., which might produce a synergistic effect and resulted in very strong inhibition of browning.

Antibrowning Activity of Mulberry Twig Extracts and Oxyresveratrol in Apple Slice Model. In the preliminary tests, apple slices were dipped into solutions of various concentrations of oxyresveratrol (0.01% to 0.2%), 4-hexylresorcinol (0.01% to 0.2%), mulberroside A (0.02% to 0.2%), and mulberry twig extracts (0.1%), then stored at 4 °C. However, repeated runs of the experiments were not able to obtain consistent antibrowning effects of the tested agents. Neither was there a clear pattern observed for their dose-dependent activities. This finding suggests that 4-hexylresorcinal or oxyresveratrol alone may not be a good antibrowning agent for fresh-cut apple slices. The most noticeable phenomenon in this test was that at high concentrations (≥0.1%) both oxyresveratrol and 4-hexylresorcinol led to an increased a^* value and thus darker apple slices. The phenomenon observed with 4-hexylresorcinol was in agreement with published literature, which reported that 4-hexy-

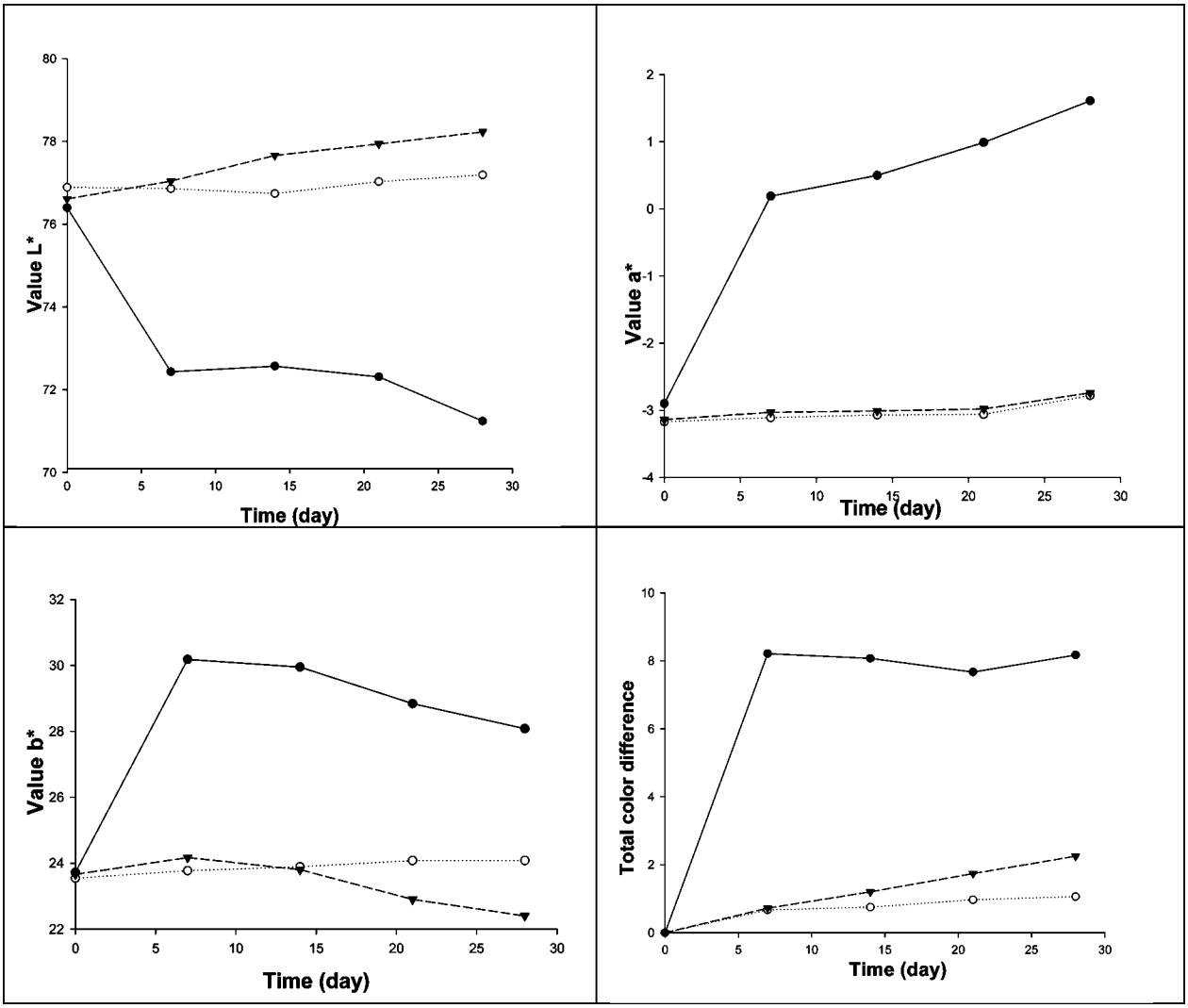

Figure 5. Reflectance measurement (L^*, a^*, b^*) and total color difference (ΔE) of apple slices treated with the following combination solutions and stored at 4 °C: - –●– control, --○-- 0.001 M 4-hexylresorcinol + 0.5 M isoascorbic acid + 0.025 M acetylcysteine + 0.05 M calcium chloride, - –▼– 0.001 M oxyresveratrol + 0.5 M isoascorbic acid + 0.025 M acetylcysteine + 0.05 M calcium chloride.

lresorcinol at high concentration led to tissue breakdown in Anjou pear slices (*25*). The present study also observed similar phenomenon for high concentration of oxyresveratrol. Although inhibitory effect of oxyresveratrol on tyrosinase has previously been reported (*27*, *28*), the mechanism of browning inhibition is still unknown. The above findings suggested that browning inhibition of oxyresveratrol was achieved possibly through a mechanism similar to that of 4-hexylresorcinol.

However, in combination with 0.5% ascorbic acid in the test solution, *Morus alba* twig extracts (0.1%), oxyresveratrol (0.01%), and 4-hexylresorcinol (0.01%) were all found to be effective in maintaining the appearance of the apple slices (kept at room temperature with open access to air) without significant color changes for at least 24 h, as shown in **Figure 4**. On the basis of the change of a^* value, oxyresveratrol, 4-hexylresorcinol, and extract B each in combination with 0.5% ascorbic acid showed better activity than 4-hexylresorcinol, oxyresveratrol, extract B, and ascorbic acid alone with significant statistical difference ($p < 0.05$) (**Table 3**). This observation also agreed with published data for 4-hexylresorcinol (*12*).

As storage period is a key factor limiting the marketing of minimally processed fruit products, various approaches to extend the storage life of fresh-cut fruits have been studied, and the combination of different chemical agents has been proven to be one of the most effective approaches. Recent studies found that the combination of 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium propionate, and 0.025 M acetylcysteine was effective in keeping Red Delicious apple slices fresh for 5 weeks at 5 °C without significant color changes (*6*). A similar combination was applied in the current study, and as shown in **Figure 5**, treated samples did not undergo any substantial browning during the entire test period. The values of total color difference (ΔE) for two tested combinations (one with oxyresveratrol and one with 4-hexylresorcinol) were significantly lower than that of the control ($p < 0.05$) for the 28 day test period. On the basis of this parameter, browning was inhibited by 87% (for the former combination) and 72% (for the latter) relative to the control. Oxyresveratrol and 4-hexylresorcinol also demonstrated comparable antibrowning effects based on the changes in L^*, a^*, and b^* values.

In summary, in both apple juice and apple slice models, it was found that oxyresveratrol and mulberry twig extract showed similar antibrowning patterns as 4-hexylresorcinol. When they are used alone, mulberry twig extracts and oxyresveratrol only showed antibrowning effects on apple juice; for fresh-cut apple slices, mulberry twig extract and oxyresveratrol need to be used together with at least ascorbic acid to achieve the antibrowning effects. The advantage of adding natural antibrowning agents

to the fresh-cut fruits and vegetables is that they are derived from edible plant tissues and thus free of regulatory constraints. Considering its widespread sources, easy extraction/isolation from these sources, and low price, oxyresveratrol shows a strong potential for practical use in the food industry. As no texture and sensory data were collected in this study, it is unknown whether, with the addition of mulberry twig extract or oxyresveartrol, the nutritional values, aroma, and flavor of apple juices and slices were changed or not, so further studies in these areas are required.

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