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# Oxyresveratrol and ascorbic acid O/W microemulsion: Preparation, characterization, anti-isomerization and potential application as antibrowning agent on fresh-cut lotus root slices

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ABSTRACT

The purpose of this study is to prepare an oxyresveratrol (Oxy) microemulsion (ME) with improved Oxy's solubility and stability and to investigate its antibrowning effects on fresh-cut lotus root slices. The formula of OxyME consisted of ethyl butyrate, Tween 80, PEG400, and water with w/w of 4%, 10.67%, 5.33%, and 80%, respectively. Encapsulating Oxy into OxyME greatly increased its solubility and stability compared with that of in water. Strong antibrowning effects were observed on fresh-cut lotus root slices treated with OxyME, even better than 4-hexylresorcinol. The addition of ascorbic acid ($V_C$) into OxyME greatly improved the Oxy stability in long-term storage and antibrowning effects on fresh-cut lotus root slices. However, the simultaneous addition of calcium chloride and $V_C$ did not obviously improve the antibrowning effects compared with the addition of $V_C$ alone. These results indicated that Oxy + $V_C$ME may be suitable as an antibrowning agent for fresh-cut vegetables.



## 1. Introduction

Appearance, flavor, texture and nutritional value of fruits and vegetables are the four important factors in customers' choice. Among them, color is the decisive factor for fruits, vegetables and crustaceans. The undesirable color change of fruits and vegetables would affect the product sensory evaluation, with the reduction of shelf life, market values and nutritional values (Fu, Li, Wang, Lee, & Cui, 2005). Usually, the underlying reason of the color change of fruits and vegetables is enzymatic browning. Enzymatic browning is primarily caused by the oxidation of phenolic compounds to generate melanin catalyzed by tyrosinase (Ibrahim et al., 2011; Yan, Li, He, Liang, & Li, 2013). Therefore, the inhibition of tyrosinase activity is an effective way for keeping the freshness of fruits and vegetables. Although some chemicals (for example sulfite) have good antibrowning effects, their applications for antibrowning on fruit and vegetables are limited due to safety reasons, limited source, poor solubility, and instability (Son, Moon, & Lee, 2000). Therefore, it is of great importance for food, pharmaceutical, and cosmetic industry to search for natural tyrosinase inhibitors which are effective, resourceful, and free of harmful side effects.

Oxyresveratrol (*trans*-2,3′,4,5′-tetrahydroxystilbene, Oxy) is a kind of polyhydroxylated stilbene naturally existed in *Moraceae*, *Liliaceae*, *Rosaceae*, *Poaceae*, *Gnetaceae*, and *Fabaceae* plants (Huang, Wang, Li, & Lin, 2000; Jo, Kim, & Lim, 2014; Shen et al., 2012; Siridechakorn et al., 2014; Xie & Bolling, 2014). It is particularly rich in many *Moraceae* plants (He & Lu, 2013; Jo et al., 2014; Zheng et al., 2010). Oxy demonstrates a broad spectrum of biological activities, such as strong tyrosinase inhibitory activity (Kim et al., 2010; Liang, Lim, Kim, & Kim, 2012; Zheng et al., 2010), anti-inflammation effects (Fang, Hsu, & Yen, 2008), antioxidant effects (Wang et al., 2011), neuroprotective effects (Chao, Yu, Ho, Wang, & Chang, 2008), etc. However, the application of Oxy in cosmetics and food industry is still limited because of its poor solubility and instability in aqueous systems, which is susceptible to oxidative discoloration and isomerization, especially when exposed to light (Silva, Figueiras, Gallardo, Nerin, & Domingues, 2014). Therefore, a suitable delivery system needs to be developed to incorporate the maximum amount of Oxy into cosmetics, functional foods and beverage products while maintaining its stability.

Microemulsion (ME) is a promising drug delivery system. It is formed spontaneously when a certain percentage of water, oil,

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surfactant and cosurfactant are admixed in an appropriate condition, which is a low viscosity, isotropic, thermodynamically stable, transparent or translucent liquid (Danielsson & Lindman, 1981; Narang, Delmarre, & Gao, 2007; Yuan, Li, Mo, & Zhong, 2006). Its particle size generally falls between 10 and 100 nm, which belongs to the colloidal system (Sasivimolphan et al., 2012). MEs possess some unique characteristics, including simple preparation, ultra-low interfacial tension, high solubilization capacity, favorable skin penetration (Muzaffar, Singh, & Chauhan, 2013; Sasivimolphan et al., 2012). Together with its favorable physical stability, it gains popularity in recent years as an ideal delivery system.

Lotus root is one of the most popular vegetables all over the world due to its good taste and abundant nutrients. However, fresh-cut lotus root is susceptible to browning during storage and processing. The approaches to inhibit browning of fresh-cut lotus root attracted a lot of attention (Jiang, Jiang, Luo, & Yu, 2014; Sun et al., 2015; Xing et al., 2010; Zhang, Yu, Xiao, Wang, & Tian, 2013). The purpose of this study is to develop a food-grade ME which was well-distributed and stable, miscible with water at infinite dilution and could effectively improve the solubility and stability of Oxy. The chemical and physical stabilities of the OxyME and Oxy + $V_C$ME were investigated as well as its antibrowning effects on fresh-cut lotus root slices.

## 2. Materials and methods

### 2.1. Materials

Tween 80, Tween 60, Tween 40, Tween 20, calcium chloride ($CaCl_2$), ethyl oleate, ethyl butyrate, DL-α-tocopherol ($V_E$), soybean oil, isopropyl myristate, caprylic capric triglyceride, polyethylene glycol 400 (PEG 400), glycerol, glycol, ethanol and methanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Ascorbic acid ($V_C$) and 4-hexylresorcinol (4-HR) were purchased from Sigma Chemical Co. (St. Louis, USA). HPLC grade methanol were purchased from J&K Scientific (New Jersey, USA). Lotus were purchased from local supermarket.

### 2.2. The solubility of Oxy in different solvents

The solubility of Oxy in various oils (ethyl butyrate, ethyl oleate, DL-α-tocopherol, soybean oil, isopropyl myristate, caprylic capric triglyceride), surfactants (Tween 20, Tween 40, Tween 60, Tween 80), cosurfactants (isopropyl alcohol, glycerol, glycol, PEG 400, ethanol) was determined by HPLC. Excess amounts of Oxy was added in 1 mL selected vehicle at 25 °C and was continuous stirred (GENIUS 3 vortex and RW 20D magnetic stirrer, IKA laboratory technology, Germany) for 24 h until dissolution equilibrium. Each sample was then centrifuged at 12,000 rpm for 20 min (Minispin Microcentrifuges, Eppendorf China Limited). The supernatant was filtered by a 0.45-μm membrane filter and quantified by HPLC after dilution with methanol. Experiments were performed in triplicate for each sample.

### 2.3. Preparation of OxyME and Oxy + $V_C$ME

The optimal formulations of OxyME were determined according to Oxy solubility in various vehicles and the phase diagrams (Supplementary files). The blank ME (BME) was prepared by mixing 4.0% ethyl butyrate, 10.67% Tween 80, and 5.33% PEG 400 together, and then by adding the required quantity of water drop by drop under the magnetic stirring to form a clear and transparent liquid by visually evaluated after equilibration. The OxyME was prepared by adding 100 mg Oxy to 10 mL to BME, stirred for 24 h to dissolve completely. The mixed solution was then centrifuged at 12,000 rpm for 20 min, the supernatant was OxyME. The Oxy and ascorbic acid ME (Oxy + $V_C$ME) was obtained by the addition of certain amount of $V_C$ to OxyME, stirred until the solution was clarified and transparent. All MEs were stored to achieve equilibrium at room temperature, at least 24 h before further investigation.

The content of Oxy in ME was quantified by HPLC after diluted 200 folds with methanol and filtered. The analyses were performed in triplicate.

### 2.4. HPLC-DAD analysis of Oxy

HPLC analyses were performed using a Shimadzu HPLC. The separation was performed in a reverse-phase GraceSmart column (4.6 μm, 2.1 × 250 mm, Ryss Tech Ltd., China) with a mobile phase of 0.1% formic acid (solvent A) and methanol (solvent B). The gradient elution was as followed: initial 20% B; 0–10 min, 20–60% B; 10–20 min, 60–100% B; 20–25 min, 100% B; 25–30 min, 100–20% B. Flow rate was 1.0 mL/min and the effluent was monitored at 307 nm with a diode array detector (SPD-M20A). Three injections were performed for each sample. The calibration curve of Oxy in the concentration range of 12.5–200 μg/mL was linear with a correlation coefficient of 0.9989.

### 2.5. Characterization of MEs

Physical properties of freshly prepared BME, OxyME and Oxy + $V_C$ME were characterized for pH and electrical conductivity using digital pH meter (Mettler Toledo, Shanghai, China) and conductivity meter (Mettler Toledo, Shanghai, China), respectively. The microstructure of MEs were observed by transmission electron microscope (TEM, JEOL JEM2100, Eindhoven, The Netherlands). The mean particle size and particle size distribution of MEs were evaluated using a photon correlation spectroscopy using Zetasizer Nano ZS (Marvern Instruments; Worcestershire, UK). The pH value, conductivity, mean particle size, and polydispersity index (PI) were determined by three independent experiments. The appearance of formulas were visually inspected for transparent, phase separation, and color. All samples were not diluted and studied at 25 °C.

### 2.6. Stability study of Oxy in MEs, water, and ethanol

In order to determine physical stability, BME, OxyME and Oxy + $V_C$ME stored at different conditions were macroscopically characterized by visual inspection, and the determinations of Oxy content, pH value, conductivity, particle size, polydispersity index were performed as described above. All samples were stored in well-closed and light protected glass bottles. Experiments were performed in triplicate for each sample.

#### 2.6.1. Acceleration storage in ME

The acceleration tests for BME, OxyME and Oxy + $V_C$ME were used the conditions by heating-cooling for three cycles. Each cycle was processed by heating at 40 °C for 48 h and cooling at 4 °C for 48 h. Physicochemical properties and Oxy content of BME, OxyME and Oxy + $V_C$ME before and after test were recorded.

#### 2.6.2. Long-term storage in ME

Long-term storage test for BME, OxyME and Oxy + $V_C$ME were static storage for 8 weeks at room temperature (10–25 °C). Physicochemical properties and Oxy content of BME, OxyME and Oxy + $V_C$ME before and after test were recorded.

#### 2.6.3. Long-term storage in water and ethanol

About 1 mg and 400 mg of Oxy (excess amounts) was added in 1 mL of water and ethanol respectively, and then the solutions were continued to stir for 24 h until the dissolution equilibrium.

**Table 1**
Physical properties, solubility determination, and stability of BME, OxyME, and Oxy + $V_C$ME under freshly prepared (25 °C), long-term, and acceleration storage.

| Conditions | Characteristics | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Transparent | Phase separation | Color | pH value | Conductivity (μS/cm) | Particle size (nm) | Polydispersity index (PdI) | Concentration (mg/mL) |
| *FPS*[a] | | | | | | | | |
| BME[d] | Found[g] | Unfound[h] | Colorless | 5.44 ± 0.01 | 133.47 ± 0.74 | 18.72 ± 0.08 | 0.35 ± 0.01 | N/D[i] |
| OxyME[e] | Found | Unfound | Light yellow | 5.32 ± 0.01 | 146.17 ± 1.32 | 26.01 ± 0.14 | 0.35 ± 0.00 | 8.55 ± 0.25 |
| Oxy + $V_C$ME[f] | Found | Unfound | Light yellow | 2.55 ± 0.00 | 927.33 ± 1.25 | 28.74 ± 0.56 | 0.31 ± 0.00 | 9.17 ± 0.41 |
| *LTS*[b] | | | | | | | | |
| BME | Found | Unfound | Colorless | 4.67 ± 0.02 | 164.10 ± 0.54 | 14.81 ± 0.09 | 0.16 ± 0.02 | N/D |
| OxyME | Found | Unfound | Deep yellow | 3.93 ± 0.02 | 183.83 ± 0.58 | 17.50 ± 0.05 | 0.24 ± 0.00 | 5.34 ± 0.89 |
| Oxy + $V_C$ME | Found | Unfound | Yellow | 1.87 ± 0.00 | 1437.00 ± 0.82 | 25.06 ± 0.23 | 0.24 ± 0.01 | 7.88 ± 0.16 |
| *AS*[c] | | | | | | | | |
| BME | Found | Unfound | Colorless | 4.42 ± 0.01 | 172.20 ± 1.55 | 12.33 ± 0.02 | 0.21 ± 0.01 | N/D |
| OxyME | Found | Unfound | Deep yellow | 3.74 ± 0.01 | 216.67 ± 2.49 | 22.53 ± 0.22 | 0.28 ± 0.02 | 7.03 ± 0.09 |
| Oxy + $V_C$ME | Found | Unfound | Light yellow | 2.15 ± 0.00 | 1153.00 ± 1.63 | 27.95 ± 0.01 | 0.26 ± 0.00 | 7.88 ± 0.27 |

Results are represented as mean ± SD (n = 3).
[a] Freshly prepared samples.
[b] Long-term storage.
[c] Acceleration storage.
[d] Blank microemulsion.
[e] OxyME oxyresveratrol-loaded ME.
[f] Oxy + $V_C$ME oxyresveratrol + ascorbic acid-loaded ME.
[g] Transparent appearance was found after centrifugation.
[h] No phase separation was found after centrifugation.
[i] N/D not detected.

Each vial was then centrifuged at 12,000 rpm for 20 min. The supernatant was storage for 6 weeks at room temperature (10–25 °C) and measured the content of Oxy in water and ethanol every 7 days, respectively.

### 2.7. Antibrowning effects of OxyME and Oxy + $V_C$ME on fresh-cut lotus slices

The lotus was cleaned, peeled, and cut into 1–1.5 cm thick slices. All samples were dipped into 200 mL solutions of different treatments for 4 min and drained. Subsequently, samples were placed in plastic Petri dishes and stored at room temperature (20 ± 2 °C) for 24 h. Visual assessments of color development in the treated samples were performed with a digital camera. The relative extent of browning was measured with a tristimulus reflectance colorimeter (Minolta CR-400 Chroma Meter). The values of $L^*$ (lightness), $a^*$ (red-green) and $b^*$ (yellow-blue) were used as parameters for monitoring the extent of browning reactions. Total color difference ($\Delta E$) was used to evaluate the antibrowning effect of different treatments, which was calculated as follows: $\Delta E = [(L_t^* - L_{initial}^*)^2 + (a_t^* - a_{initial}^*)^2 + (b_t^* - b_{initial}^*)^2]^{0.5}$. Measurements were made immediately following each treatment and at timed intervals of 0, 3, 6, 12, 9, and 24 h thereafter. All experiments were performed nine times (2 slices × 9). Test solutions used for the above samples included BME, 0.05% $V_C$, 0.05% $CaCl_2$, 0.01% 4-HR (4-hexylresorcinol), 0.01% OxyME, 0.01% 4-HR + 0.05% $V_C$, 0.01% Oxy + 0.05% $V_C$ME, 0.01% 4-HR + 0.05% $V_C$ + 0.05% $CaCl_2$, 0.01% Oxy + 0.05% $V_C$ME + 0.05% $CaCl_2$.

### 2.8. Statistical analysis

All experiments in the study were performed at least three times and the data were reported as mean ± SD. The statistical significance among different treatments in each individual experiment was compared by ANOVA using SPSS 13.0 (SPSS Inc, Chicago, IL, USA) statistical-analysis system. Values of $p < 0.05$ were considered to be significantly different.

## 3. Results and discussion

### 3.1. Solubility study

In this study, the ME formula was selected on the basis of their ability to form ME as well as the solubility of Oxy. The maximum loading of Oxy in different vehicles was determined by HPLC, and results are shown in Table S1 (Supplementary material). From Table S1, it could be found that Oxy exhibited highest solubility in ethyl butyrate compared with other oils, up to 71.87 ± 2.33 mg/mL. In addition, Tween 80 and PEG400 showed maximal solubilizing capacity for Oxy, up to 168.90 ± 2.09 mg/mL and 445.37 ± 6.07 mg/mL, respectively. Based on the above solubility results, ethyl butyrate, Tween 80, and PEG 400 that showed maximum solubility for Oxy were selected for further OxyME formulation studies.

### 3.2. Preparation of OxyME and Oxy + $V_C$ME and content determination of Oxy in ME

Firstly, ethyl butyrate, Tween 80, and PEG 400 were found to exhibit better solubility for Oxy than other selected oils, surfactants, and cosurfactants through solubility study, therefore they were selected for further OxyME formulation studies. Secondly, the appropriate ratios of oils, surfactants, and cosurfactants to form stable, clarified and transparent MEs were usually determined by the construction of pseudo-ternary phase diagrams (Supplementary material). When the weight ratio of ethyl butyrate and the mixture of Tween 80 and PEG 400 (Tween 80: PEG 400, 2:1) was 1:4, the mixture of ethyl butyrate, Tween 80, and PEG 400 formed into stable, clarified and transparent MEs which could be diluted with water infinitely. Thirdly, MEs could be separated into W/O, bicontinuous (B.C) or O/W type according to the correlation curve of electrical conductivity and water content (Mo, Zhong, & Zhong, 2000). MEs belong to O/W type when the content of water was over 77%, therefore, the content of water was determined as 80% in this study. Based on the above results, optimized formula of OxyME consisted of 4% w/w of ethyl butyrate, 10.67% w/w of Tween 80, 5.33% w/w of PEG400, and 80% w/w of water. The

solubility of the Oxy in OxyME was increased about 22 times compared with its solubility in water, up to 8.55 ± 0.25 mg/mL. Incorporating $V_C$ into OxyME further slightly improved Oxy's solubility in ME, up to 9.17 ± 0.41 mg/mL (Table 1).

### 3.3. Characterization of MEs

MEs were characterized in terms of visual inspection (Fig. 1-A), droplet size distribution (Table 1, Fig. 2-A), polydispersity index (PdI) (Table 1), transmission electron microscopy (TEM, Fig. 2-A), pH values, and conductivity (Table 1).

The appearance of BME was colorless, clarified, transparent liquid with good fluidity, whereas OxyME and Oxy + $V_C$ME were light yellow clarified, transparent liquids with good fluidity (Fig. 1-A).

The mean particle sizes of BME, OxyME, and Oxy + $V_C$ME were 18.72 ± 0.05 nm, 26.01 ± 0.14 nm, and 28.74 ± 0.56 nm (Table 1), respectively. The incorporation of Oxy and $V_C$ in MEs did not significantly influence size of the ME droplets. In addition, the values of polydispersity indices (PdI) were 0.35 ± 0.01, 0.35 ± 0.00, and 0.31 ± 0.00 for BME, OxyME, and Oxy + $V_C$ME, respectively, indicating the narrow size distribution of MEs (Joshi & Patravale, 2006). TEM analysis of BME, OxyME, and Oxy + $V_C$ME showed the presence of droplets with a mean diameter around 12–29 nm (Fig. 2-A), coherent with particle sizes measured by dynamic light scattering.

The pH values of BME and OxyME were 5.44 ± 0.01 and 5.32 ± 0.01, respectively, a slight decrease after incorporation of Oxy into ME. However, the addition of $V_C$ into OxyME significantly reduced the pH value of the system, down to 2.55 ± 0.00, which may due to the increase of hydrogen ion released from $V_C$.

The conductivities of BME, OxyME, and Oxy + $V_C$ME were 133.47 ± 0.74, 146.17 ± 1.32, and 927.33 ± 1.25 μS/cm, respectively. Similarly, the incorporation of $V_C$ into ME led to the great increase of conductivity value, which may be due to the increase number of mobile ions. Furthermore, in the case of high conductivity, the MEs still belonged to O/W type (Baboota et al., 2011; Baroli, 2000), and the incorporation of Oxy and $V_C$ did not change the structure of the system.

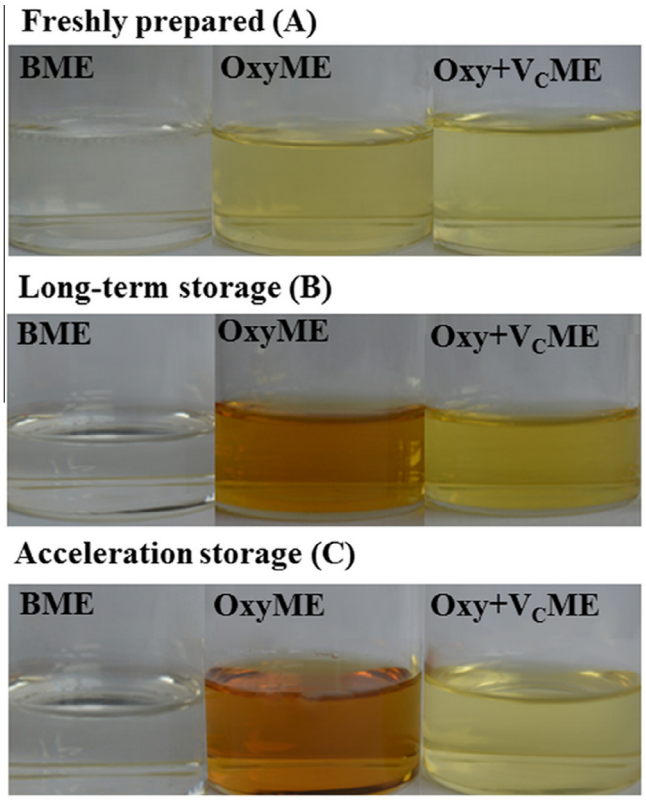


**Fig. 1.** Visual appearance of BME, OxyME, Oxy + $V_C$ME for freshly prepared (A), long-term storage (B), and acceleration storage (C).

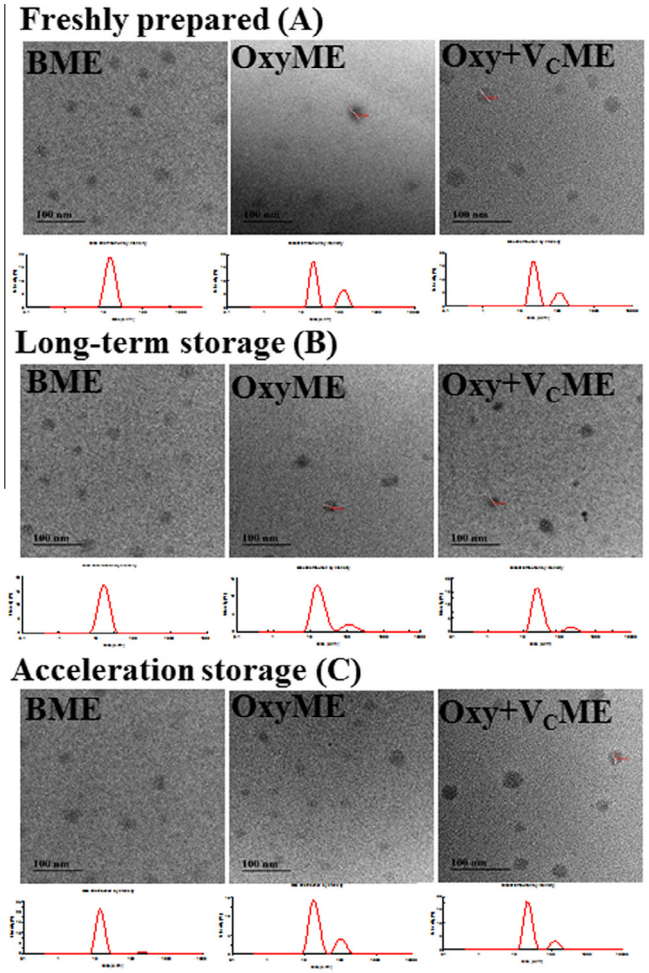


**Fig. 2.** Morphology and particle size distribution of BME, OxyME, Oxy + $V_C$ME for freshly prepared (A), long-term storage (B), and acceleration storage (C).

### 3.4. Stability study

Isomerization from the *trans* to the *cis* form is often found in some stilbene derivatives. For example, resveratrol is an extremely photosensitive compound, it may be chemically degraded when exposed to elevated temperatures, pH changes, ultraviolet light, or certain types of enzymes (Davidov-Pardo & McClements, 2014). Most of the *trans*-resveratrol (80–90%) in solution was converted to *cis*-resveratrol when exposed to light for 1 h (Vian, Tomao, Gallet, Coulomb, & Lacombe, 2005). Similar problem was found for oxyresveratrol. Some of *trans*-oxyresveratrol will convert to *cis*-oxyresveratrol in polar solvents (such as water, methanol or ethanol) when exposed to light (Fig. 3-A) and therefore leads to the decrease of its tyrosinase inhibitory activity and antibrowning capability. Therefore, MEs were developed to improve the stability of Oxy and prevent this isomerization (Fig. 3-B).

After acceleration and long-term storage, BME, OxyME, and Oxy + $V_C$ME were still transparent and clear by visual observation (Fig. 1-B ~ C). However, the color of OxyME significantly changes, from the light yellow to deep yellow, whereas the color of Oxy + $V_C$ME changed from light yellow to yellow. The discoloration of OxyME and Oxy + $V_C$ME may be due to the oxidation of Oxy

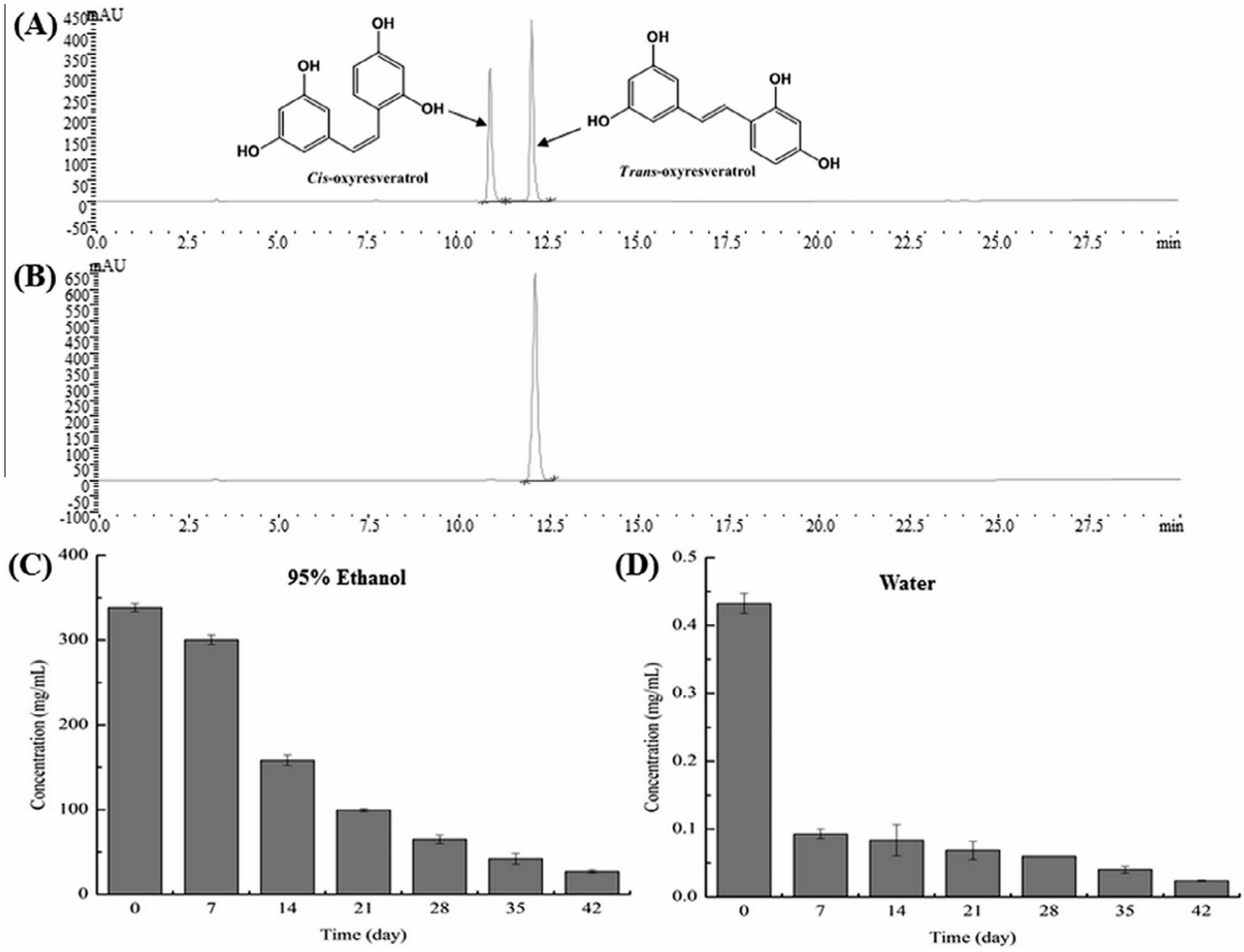


**Fig. 3.** HPLC chromatograph of Oxy in polar solvent (A) and in ME (B), the content change of Oxy in 95% ethanol (C) and water (D) at room temperature during six week storage.

(Park et al., 2014; Silva et al., 2014). Morphology of MEs was found to have no significant changes after acceleration and long-term storage (Fig. 2-B ~ C), spherical particles and uniform distribution were kept.

After the acceleration storage, the pH values of BME, OxyME and Oxy + $V_C$ME had a little decrease compared with freshly prepared samples, about 1.02 units, 1.58 units, and 0.4 units, respectively. The conductivity had greatly increased, up to 38.73 μS/cm, 70.50 μS/cm and 225.67 μS/cm of BME, OxyME and Oxy + $V_C$ME, respectively. The content of Oxy in OxyME and Oxy + $V_C$ME was decreased to 7.03 ± 0.09 mg/mL and 7.88 ± 0.27 mg/mL, respectively. The mean particle sizes of MEs slightly decreased to 12.33 ± 0.02, 22.53 ± 0.22, 27.95 ± 0.01 nm. In long-term storage, compared with freshly prepared samples, the pH values had also little decreased, up to 0.77 units, 1.39 units and 0.68 units for BME, OxyME and Oxy + $V_C$ME, respectively. The conductivity was greatly increased as well, about 30.63 μS/cm, 37.66 μS/cm and 509.67 μS/cm of BME, OxyME and Oxy + $V_C$ME, respectively. The content of Oxy significantly decreased, especially for OxyME, from 8.55 ± 0.25 mg/mL down to 5.34 ± 0.89 mg/mL, reduced about 37.54%. However, the addition of $V_C$ into the OxyME greatly improved the stability of Oxy, the content of Oxy only reduced 14.07% in Oxy + $V_C$ME. The mean particle sizes of MEs slightly decreased to 14.81 ± 0.09, 17.50 ± 0.05, 25.06 ± 0.23 nm, respectively.

The Oxy content in water and ethanol during six weeks of storage at room temperature is shown in Fig. 3-C ~ D. In the process of storage, it was found that, with the increase of storage time, the samples were precipitated and the content of Oxy decreased gradually in solutions. In 95% ethanol, the Oxy content decreased massively from 338.42 ± 4.80 mg/mL to 21.57 ± 8.15 mg/mL after 42 days of storage (6 weeks), up to 93.67% loss of Oxy (Fig. 3-C). In water, and the content of Oxy decreased rapidly to 0.09 ± 0.01 mg/mL after 7 days of storage, reaching to 79.07% loss of Oxy. After 42 days of storage, the loss of Oxy was even up to 94.42% (Fig. 3-D), whereas the loss of Oxy in MEs was only 37.54% after 8 weeks storage.

Taken together, the usage of ME technology not only greatly increased the solubility of the Oxy, but also greatly improved its stability, especially in the long-term storage. The incorporation of $V_C$ into OxyME helped to further improved Oxy stability in long-term storage.

### 3.5. Antibrowning effects of OxyME and Oxy + $V_C$ME on fresh-cut lotus slices

The tristimulus reflectance colorimeter (Minolta CR-400 Chroma Meter) was often used to demonstrate antibrowning effects of different treatment solutions based on the parameters $\Delta E$ (total color difference), $a^*$ (red-green), $L^*$ (lightness), and $b^*$ (yellow-blue). These parameters have been usually used as parameters for measuring the extent of browning reactions in fruits and vegetables. An increase in $\Delta E$ or $a^*$ and a decrease in $L^*$ mean the occurrence of browning. Among them, $\Delta E$ and $L^*$ values were usually used to monitor the browning process for fresh-cut lotus root slices (Jiang et al., 2014; Sun et al., 2015; Zhang et al.,

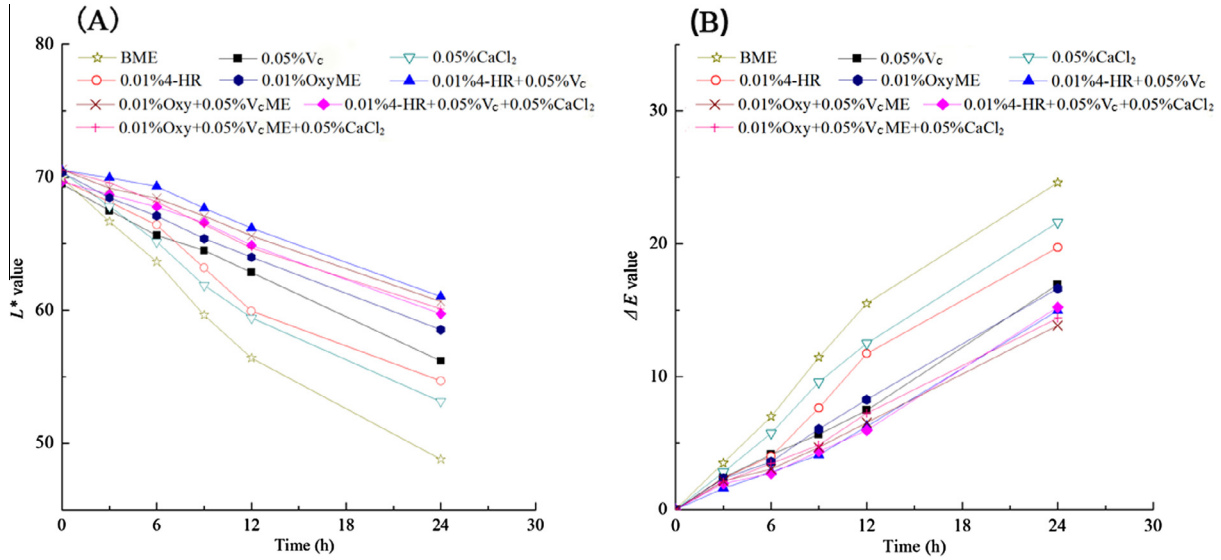


**Fig. 4.** Reflectance measurement of $L^*$ (A) and $\Delta E$ (B) values of fresh-cut lotus root slices treated with the difference tested solutions and stored at room temperature in 24 h.

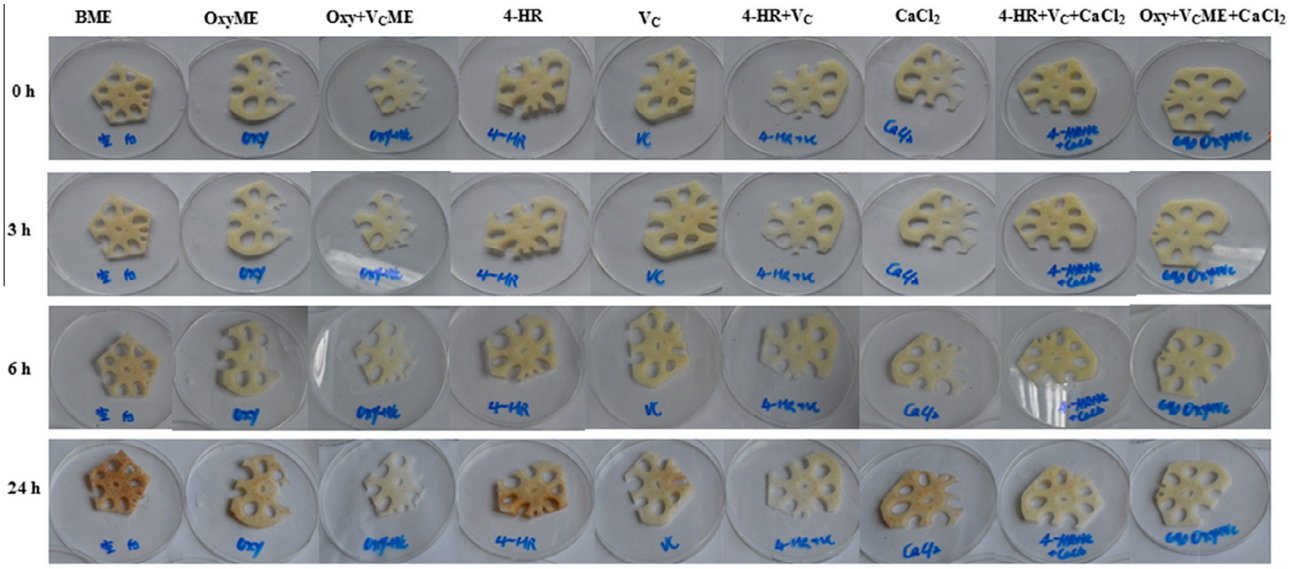


**Fig. 5.** Visual appearance of fresh-cut lotus root slices treated with the difference tested solutions and stored at room temperature in 0, 3, 6, and 24 h.

2013). Moreover, studies showed that plant extracts or compounds combined with $V_C$ could greatly improve their antibrowning effects (Li, Cheng, Cho, He, & Wang, 2007; Luo & Barbosa-Canovas, 1997; Rojas-Grau, Soliva-Fortuny, & Martín-Bellsos, 2008). Meanwhile, some studies indicated that $CaCl_2$ was an ideal browning inhibitor and it was effective in preventing the decrease of nutrient contents and maintaining the appearance of some fruits and vegetables (Kulkarni & Vijayanand, 2012; Wu, Zhang, & Wang, 2011).

In this study, $\Delta E$ and $L^*$ values were chosen as parameters for evaluation of the antibrowning activity of OxyME and Oxy + $V_C$ME on fresh-cut lotus slices, and the degree of browning was usually associated with the decrease of $L^*$ value and the increase of $\Delta E$ value, together with visual observation. The test solutions of 0.05% $V_C$, 0.05% $CaCl_2$, 0.01% 4-HR, 0.01% OxyME, 0.01% 4-HR + 0.05% $V_C$, 0.01% Oxy + 0.05% $V_C$ME, 0.01% 4-HR + 0.05% $V_C$ + 0.05% $CaCl_2$, 0.01% Oxy + 0.05% $V_C$ME + 0.05% $CaCl_2$ were all found to be effective in maintaining the appearance of the lotus root slices (kept at room temperature with open access to air).

On the basis of the change of $L^*$ values (Fig. 4-A), BME almost did not show antibrowning effects on lotus root slices even at 3 h in room temperature (25 °C), as shown in Fig. 4. 0.01% OxyME showed stronger antibrowning effects on lotus root slices than 0.01% 4-HR and 0.05% $V_C$. The treatment of 0.01% Oxy + 0.05%$V_C$ME and 0.01% 4-HR + 0.05% $V_C$ showed the strongest antibrowning effects on lotus root slices in 24 h among all treatments with no significant difference according to the change of $L^*$ values, suggesting that Oxy + $V_C$ME and 4-HR + $V_C$ could better inhibit the happening of browning for lotus root slices better than OxyME and 4-HR alone. It was reported that plant extracts or compounds usually needed to be used in combination with $V_C$ to exhibit better antibrowning effects (Li et al., 2007; Luo and Barbosa-Canovas, 1997; Rojas-Grau et al., 2008). The results of this study also confirmed the addition of $V_C$ into the treatments greatly increased the antibrowning effects of OxyME and 4-HR on lotus root slices. Although some studies suggested that $CaCl_2$ was an ideal browning inhibitor used to maintain the appearance of some fruits and

vegetables (Kulkarni and Vijayanand, 2012; Wu et al., 2011). However, the results in this study showed that the treatment of 0.05% $CaCl_2$ alone almost did not show antibrowning effects even at 3 h in room temperature. Treatments of 0.01% Oxy + 0.05% $V_C$ME and 0.01% 4-HR + 0.05% $V_C$ combined with 0.05% $CaCl_2$ decreased their antibrowning effects, indicating that $CaCl_2$ was not an effective browning inhibitor for lotus root slices. The results of $\Delta E$ values (Fig. 4-B) displayed the same trend with the results of $L^*$ values. The above findings suggested that browning inhibition of OxyME and Oxy + $V_C$ME were achieved possibly through a mechanism similar to that of 4-HR. These results suggested that OxyME and Oxy + $V_C$ME may be a potential antibrowning agents for lotus root slices. The visual assessment agreed with colorimetric measurements (Fig. 5).

## 4. Conclusions

In this study, OxyME and Oxy + $V_C$ME were prepared and characterized, and their chemical and physical stabilities and antibrowning effects on fresh-cut lotus root slices were also evaluated for the first time. The results suggested that the incorporation of Oxy into ME greatly improved its solubility and stability. The incorporation of $V_C$ into OxyME helped to further improve the stability of Oxy. Both OxyME and Oxy + $V_C$ME remained good physicochemical stability after acceleration and long-term storage. Both of them showed strong antibrowning effects on fresh-cut lotus root slices which were demonstrated by the parameters $L^*$ and $\Delta E$ values. Taken together, it is suggested Oxy + $V_C$ME had great potential as antibrowning agents for food industry in terms of the enhanced efficacy and stability. Therefore, they could be employed as antibrowning agents to extend the shelf-life of fresh-cut lotus root slices.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.07.095.

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