



# Stability of $\beta$ -carotene microcapsules with Maillard reaction products derived from whey protein isolate and galactose as coating materials\*

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**Abstract:** The stability of  $\beta$ -carotene microcapsules using Maillard reaction products (MRPs) derived from whey protein isolate (WPI) and galactose as coating materials, was studied under the varying environmental conditions of temperature, pH, air, incandescent light, and ultraviolet (UV) light. Scanning electron microscopy showed that microcapsules prepared by WPI-galactose MRPs displayed a smooth and less concave-convex surface and that the particle size ( $D_{50}$ ) of the microcapsules made with WPI-galactose MRPs was smaller than those made with WPI-galactose mixture. The storage stability of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs was remarkably better than that of  $\beta$ -carotene microencapsulated in the WPI-galactose mixture and that of  $\beta$ -carotene crystal, in respect of temperature, pH, air, incandescent light, and UV light measurements. When the storage temperature was increased from 5 to 105 °C, the retention rate of  $\beta$ -carotene microcapsules significantly decreased ( $P < 0.05$ ). When pH values were increased from 1 to 12, the  $\beta$ -carotene retention rate of the microcapsules significantly increased and afterward decreased. Compared with the retention rate of  $\beta$ -carotene microencapsulated in a WPI-galactose mixture, the retention rate of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs was at a maximum between pH 8 and 9. Under the actions of air, incandescent light, and UV light, the retention rates of  $\beta$ -carotene microcapsules in WPI-galactose MRPs and WPI-galactose mixture, as well as in  $\beta$ -carotene crystal, decreased significantly as the storage time increased ( $P < 0.05$ ). Therefore, the use of WPI-galactose MRPs as coating materials can aid in improving the storage stability of  $\beta$ -carotene microcapsules.

**Key words:** Maillard reaction products (MRPs); Whey protein isolate (WPI);  $\beta$ -Carotene; Microcapsule; Stability  
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## 1 Introduction

$\beta$ -Carotene belongs to four terpenoid compound categories, which is an orthorhombic 6 crystal and generally exists in animals, plants, fungi, bacteria, and algae (Masek *et al.*, 2015).  $\beta$ -Carotene is the most abundant carotenoid in nature and has strong vitamin

A activity. There are a total of nine conjugated double bonds in the molecular structure of  $\beta$ -carotene, which result in its unsaturated structures and active chemical properties, and leave it prone to oxidative deterioration and isomerization reactions in light and heat conditions. Exposure to light, heat, and oxygen should therefore be avoided during the storage process of  $\beta$ -carotene. As a fat-soluble substance, the natural  $\beta$ -carotene is slightly soluble in edible oil, and only soluble in a small number of organic solvents, such as hexane, acetone, chloroform (Gardner *et al.*, 2010). An important method for solving this problem is putting  $\beta$ -carotene into microcapsule powder products, where  $\beta$ -carotene is embedded, to avoid

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contact with air, in an amorphous form of core materials in the microcapsule powder, which effectively enhances the bioavailability of  $\beta$ -carotene in the human body. Encapsulation is extensively used in various processing field (Zhu *et al.*, 2015).

Maillard reaction products (MRPs) from various food proteins have strong antioxidant activity (Brands and van Boekel, 2003), good emulsifying properties (Akhtar and Dickinson, 2003), and film forming properties (Wang *et al.*, 2014), which are used to make safe functional antioxidant wall materials for microcapsules. As yet, however, MRPs derived from food protein and reducing sugar have not been used as coating materials in the preparation of  $\beta$ -carotene microcapsules, and instead coating materials mainly consist of malt dextrin, chitosan and alginic acid salt, poly hydroxy butyric acid, pentyl ester, and modified starch (Loksuwan, 2007; Donhowe *et al.*, 2014). Galactose is highly reactive with various proteins in the Maillard reaction. Introducing the whey protein isolate (WPI)-galactose MRPs makes it possible for processors to control the release of core material and to be useful for retarding its oxidation.

Light, oxygen, heat, and other environmental factors have a strong influence on the stability of  $\beta$ -carotene (Boon *et al.*, 2008; 2009). At 70 °C, the degradation rates of  $\beta$ -carotene in emulsions prepared with WPI-pectin glycosylated products were far less than those in emulsions prepared with a WPI-pectin mixture (Xu *et al.*, 2012). The  $\beta$ -carotene retention rates of a  $\beta$ -carotene emulsion containing WPI increase gradually in a pH range of 3 to 7 at 55 °C (Xu *et al.*, 2013).  $\beta$ -Carotene microcapsules encapsulated with 12 dextrose equivalent (DE) hydrolyzed starch exhibited the highest stability in the presence of ultraviolet (UV) light at (25±2) °C (Xu *et al.*, 2013). However, the effects of environmental changes on the storage stability of  $\beta$ -carotene microcapsules prepared using MRPs as wall materials have not been clarified. Therefore, in this study,  $\beta$ -carotene microcapsules were prepared using WPI-galactose MRPs as a coating material due to its high antioxidant and functional properties.  $\beta$ -Carotene microcapsules were characterized by their surface morphology and size distribution. The influence on the storage stability of these  $\beta$ -carotene microcapsules of five types of environmental changes that included temperature, pH, air, incandescent light, and UV light, was then investigated.

## 2 Materials and methods

### 2.1 Materials

WPI (90% protein content) was purchased from the Hilmar Cheese Company, Inc., USA.  $\beta$ -Carotene with a purity of 98% was purchased from Shanghai Chemical Technology Co., Ltd. (Shanghai, China). Vegetable oil with a  $\beta$ -carotene content of 30% was purchased from Zhejiang Pharmaceutical Co., Ltd. (Hangzhou, China). Galactose and hexane were purchased from Shanghai Biological Technology Co., Ltd. (Shanghai, China). Tween-80 and glycerol monostearate were purchased from Tianjin Guangfu Chemical Research Centre (Tianjin, China). All other chemicals and reagents used in this study were of analytical grade.

### 2.2 Preparation of $\beta$ -carotene microcapsules

The WPI and galactose were dissolved in deionized water. WPI (30 g/L) reacted with 30 g/L galactose at 95 °C with an initial pH value of 9 in sealed screw-top glass tubes. After 3 h of heat treatment, the reaction solution was cooled immediately in ice water as coating material solutions. Subsequently, a  $\beta$ -carotene suspension was added to the coating material solutions in a ratio of 15:100 (w/w) along with 24 g/L of Tween-80 and 6 g/L of glycerol monostearate. The mixture was then homogenized to obtain an aqueous emulsion and immediately fed to the spray-dryer (B290, Buchi Laboratory Instruments Co., Switzerland). The spray-dried powders were collected, kept in plastic bags wrapped with aluminum foil, and stored in desiccators containing silica gel at room temperature.

### 2.3 Surface morphology observation

The morphology of the microcapsule samples was observed using a scanning electron microscope (Hitachi S-3400, Japan) at an accelerating voltage of 5 kV and 3000× magnification. Before using the scanning electron microscope, the samples were ground slightly and coated with gold to a thickness of 15 nm using an ion sputter (Hitachi E-1010, Japan).

### 2.4 Particle size measurement

The particle sizes of the sample solutions were determined using an HYL-1076 instrument (Dan-Dong Hylology Technology Co., Ltd., Liaoning,

China). Stokes-Einstein equation was used to calculate the particle sizes, which are reported as  $D_{50}$ , standing for the average particle size of the samples.

## 2.5 Determination of $\beta$ -carotene content

To measure the initial  $\beta$ -carotene content and  $\beta$ -carotene retention during storage, the methodology described by Desobry *et al.* (1997) was used, with small modifications. A total of 0.20 g of the sample was dispersed in 3 ml of water and then 20 ml of hexane was added. This mixture was ultrasonicated for 60 min at 25 °C and centrifuged at 3000g for 3 min. Absorbance of the supernatants was determined at 450 nm with a UV-visible spectrophotometer (UT-1800, Shimadzu Corporation, Japan). A calibration curve was obtained with  $\beta$ -carotene in hexane (0 to 20 g/ml). The  $\beta$ -carotene content in the microcapsules was calculated according to the calibration curve equation ( $C=9.647A+0.6329$ ,  $C$  representing the  $\beta$ -carotene concentration,  $A$  representing the absorbance,  $R^2=0.9914$ ).

## 2.6 $\beta$ -Carotene retention rate of microcapsules

The total  $\beta$ -carotene content of the microcapsules was determined both at the start of the experiment and after a period of storage. The retention rate of the microcapsules was calculated according to the following formula: retention rate (%)=(total  $\beta$ -carotene content of microcapsules)/(total initial  $\beta$ -carotene content of microcapsules) $\times$ 100%.

## 2.7 Experimental procedure

### 2.7.1 Effect of temperature on the stability of the microcapsules

The  $\beta$ -carotene crystal,  $\beta$ -carotene microcapsule samples prepared with WPI-galactose MRPs and WPI-galactose mixture as wall materials were placed into amber glasses and sealed in the dark at 5, 10, 15, 25, 35, 45, 55, 65, 75, 85, 95, and 105 °C for 6 h. After treatment with the different temperatures, the  $\beta$ -carotene concentrations of the microcapsules were determined and the  $\beta$ -carotene retention rate was calculated.

### 2.7.2 Effect of pH on the stability of the microcapsules

The  $\beta$ -carotene microcapsule samples were dissolved with deionized water and their pH values were adjusted to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

After treatment with different pH values at 25 °C for 3 h, the  $\beta$ -carotene concentration of each microcapsule sample was determined and the  $\beta$ -carotene retention rate was calculated.

The  $\beta$ -carotene microcapsule samples were dissolved with deionized water at a concentration of 70 mg/ml and their pH values were adjusted to 4 and 7 at 4, 10, and 25 °C. The  $\beta$ -carotene retention rates were measured every 20 h over a period of 5 d.

### 2.7.3 Effect of air on the stability of microcapsules

The  $\beta$ -carotene microcapsule samples were placed in a dark thermostatic open environment at 4, 10, and 25 °C for 5, 10, 15, 20, 25, and 30 d. After air treatment, the  $\beta$ -carotene concentrations of the microcapsules were determined and the  $\beta$ -carotene retention rates were calculated.

### 2.7.4 Effects of incandescent light and ultraviolet light on the stability of microcapsules

The  $\beta$ -carotene microcapsule samples prepared with WPI-galactose MRPs and WPI-galactose mixture were placed into transparent glasses and sealed at 4, 10, and 25 °C under an incandescent light (25 W), which was positioned parallel to the samples at a distance of 34 cm. After treatment with the incandescent light, the samples'  $\beta$ -carotene concentrations and retention rates were determined.

A further  $\beta$ -carotene microcapsule samples were placed into transparent glasses and sealed at 25 °C under a UV light (100 W), which was positioned parallel to them at a distance of 50 cm. After 120 h of UV light treatment, the  $\beta$ -carotene concentrations were measured and retention rates were calculated.

## 2.8 Statistical analysis

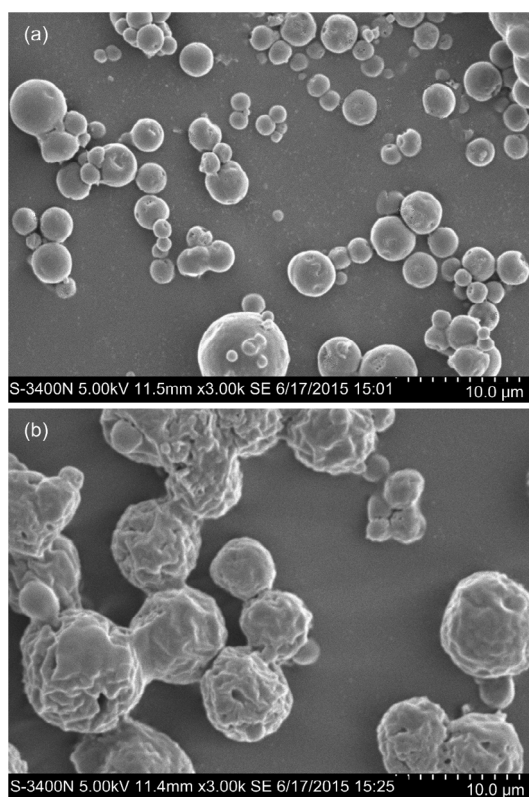
Three independent trials were carried out on  $\beta$ -carotene microcapsules prepared with MRPs derived from WPI and galactose as wall materials. Determinations of the microcapsules'  $\beta$ -carotene retention rates and particle sizes were run in triplicate. The SPSS 17.0 statistical software was used to carry out a one-way analysis of variance (ANOVA) in order to determine the significance of the main effects. Experimental results were expressed as means with standard deviation (SD). Significant differences ( $P<0.05$ ) among means were identified using Duncan's multiple range tests and independent  $t$ -tests. For the

standard deviation of the mean (SDM) analysis, the experiments were carried out in triplicate.

### 3 Results and discussion

#### 3.1 Surface morphology of $\beta$ -carotene microcapsules

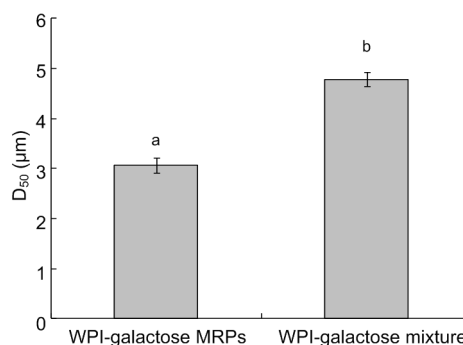
Both the  $\beta$ -carotene microcapsules using WPI-galactose MRPs as coating materials and those using the WPI-galactose mixture were prepared by spray drying. The surface morphology of the microcapsules was observed using scanning electron microscope (Fig. 1). Those that used the WPI-galactose mixture produced a rough and more concave-convex surface, which may be due to the rapid shrinking of wall materials during the cooling process after high temperature spray drying (Rosenberg and Young, 1993). In comparison, the microparticles prepared with the WPI-galactose MRPs displayed a smooth and less concave-convex surface due to their good film forming properties and antioxidant activity.



**Fig. 1** Scanning electron microscope photographs of spray-dried microcapsules encapsulating  $\beta$ -carotene using WPI-galactose MRPs (a) and WPI-galactose mixture (b) as coating materials

#### 3.2 Particle sizes of $\beta$ -carotene microcapsules

The particle sizes ( $D_{50}$ ) of both the WPI-galactose MRP- and WPI-galactose-prepared spray-dried microcapsules are shown in Fig. 2.  $D_{50}$  of the  $\beta$ -carotene microcapsules prepared by WPI-galactose MRPs was smaller than that of those prepared with the WPI-galactose mixture. This may be related to the stability of the emulsion prior to spray drying. WPI-galactose MRPs have excellent emulsifying activity, which could form a more stable emulsion under the same preparation conditions. Emulsion stability could optimize the physical and chemical properties of microcapsules (Tan *et al.*, 2005).



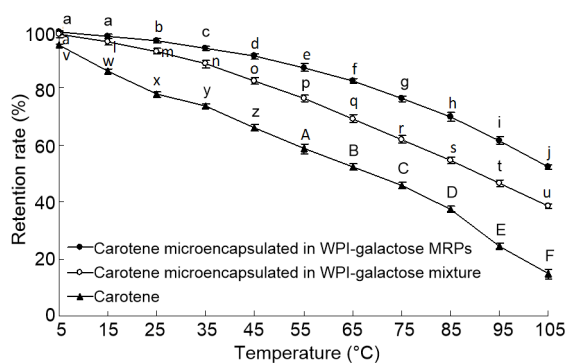
**Fig. 2** Particle sizes ( $D_{50}$ ) of spray-dried microcapsules using WPI-galactose MRPs and WPI-galactose mixture as coating materials

Error bars represent the standard deviation of the mean of triplicate experiments. Values with different letters are significantly different ( $P < 0.05$ )

#### 3.3 Effect of temperature on the degradation of $\beta$ -carotene microcapsules

Fig. 3 shows the degradation of  $\beta$ -carotene and  $\beta$ -carotene microencapsulated in both WPI-galactose MRPs and a WPI-galactose mixture when stored under different temperatures. The  $\beta$ -carotene retention rates of all three significantly decreased as temperatures rose from 5 to 105 °C ( $P < 0.05$ ). However, it can be seen that the retention rate of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs was remarkably higher than that of both the  $\beta$ -carotene microencapsulated in the WPI-galactose mixture and the  $\beta$ -carotene crystals ( $P < 0.05$ ). For example, on reaching a temperature of 105 °C, the retention rate of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs had decreased to only 51.79 %, compared with the decrease in  $\beta$ -carotene retention rate of the

WPI-galactose mixture to 38.24 % and that of the  $\beta$ -carotene crystals to 14.92%. As the temperature increased, structural changes occurred in the wall material; gaps in the microcapsule membrane widened, enlarging structural defects in the wall material and resulting in the deterioration of its density, the retention capability of the microcapsules thus reduced accordingly. Under high temperature conditions, microencapsulated  $\beta$ -carotene loss was much more moderate compared with the loss from the  $\beta$ -carotene crystals. However, high temperatures causing wall material degeneration and melting meant that microencapsulated  $\beta$ -carotene was also lost to an extent and it can therefore be concluded that microencapsulated  $\beta$ -carotene has better storage stability at a low temperature.



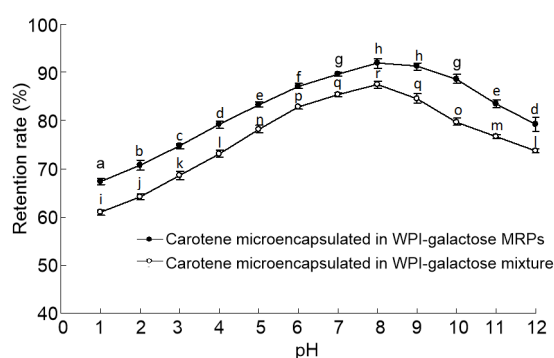
**Fig. 3** Degradation of  $\beta$ -carotene crystal and  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and WPI-galactose mixture stored under different temperatures Error bars represent the standard deviation of the mean of triplicate experiments. Values with different letters are significantly different ( $P<0.05$ )

### 3.4 Effect of pH on the degradation of $\beta$ -carotene microcapsules

#### 3.4.1 Effect of different pH values on the degradation of $\beta$ -carotene microcapsules

The pH values of aqueous systems have a strong impact on the stability of microcapsules (Gu *et al.*, 2009). Fig. 4 shows the effects of different pH values of the aqueous system on the stability of  $\beta$ -carotene microcapsules under dark and sealed conditions. The retention rate of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs was higher than that of the WPI-galactose mixture as the pH value increased from 1 to 12 ( $P<0.05$ ). Furthermore, as the pH in-

creased from 1 to 12, the retention rates of the  $\beta$ -carotene microencapsulated in both the WPI-galactose MRPs and the WPI-galactose mixture first increased and then decreased ( $P<0.05$ ). For instance, the retention rate of  $\beta$ -carotene microencapsulated in the WPI-galactose MRPs reached its highest at pH 8 and 9, corresponding to 91.47% and 91.52%, respectively. When the pH value ranged from 9 to 12, the retention rate of  $\beta$ -carotene microencapsulated in the WPI-galactose MRPs became significantly lower ( $P<0.05$ ).

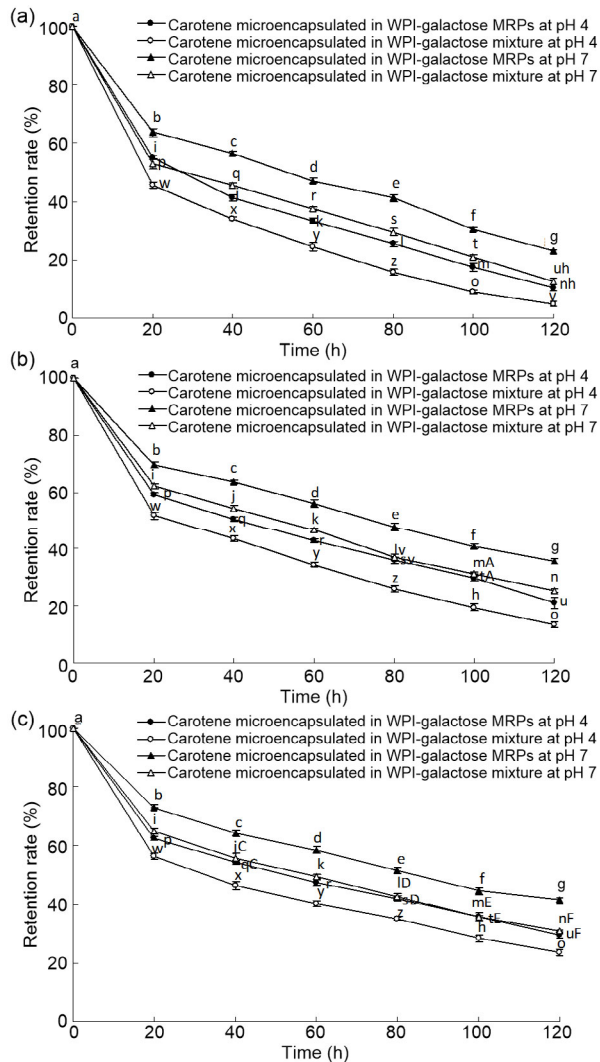


**Fig. 4** Degradation of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and WPI-galactose mixture stored at different pH values of the aqueous system Error bars represent the standard deviation of the mean of triplicate experiments. Values with different letters are significantly different ( $P<0.05$ )

#### 3.4.2 Degradation changes of $\beta$ -carotene microcapsules at pH 4 and 7

Acidic and neutral systems are used extensively in the food industry so this study considered the degradation of  $\beta$ -carotene microcapsules at different storage temperatures in such systems. Degradation changes of  $\beta$ -carotene microencapsulated in both the WPI-galactose MRPs and the WPI-galactose mixture, stored at 25 °C in an aqueous system of pH 4 and 7, are shown in Fig. 5a. When  $\beta$ -carotene microcapsules were stored at 25 °C in an aqueous system of pH 4 and 7, the retention rate of  $\beta$ -carotene microencapsulated in the WPI-galactose MRPs was higher than it was in the WPI-galactose mixture ( $P<0.05$ ). The  $\beta$ -carotene retention rates of both the WPI-galactose MRPs and the WPI-galactose mixture significantly decreased at 25 °C in the aqueous systems of pH 4 and 7 with increasing storage time ( $P<0.05$ ). For instance, the

$\beta$ -carotene retention rates of both the WPI-galactose MRPs and the WPI-galactose mixture microcapsules, when stored at pH 4 and 25 °C for 120 h, decreased to 9.34% and 5.29%, respectively. When stored at pH 7 and 25 °C for 120 h, their retention rates decreased to 21.76% and 15.29%, respectively.



**Fig. 5** Degradation changes of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and WPI-galactose mixture stored at pH 4 and 7

(a)  $\beta$ -Carotene microcapsules were placed at 25 °C in the aqueous systems of pH 4 and 7. (b)  $\beta$ -Carotene microcapsules were placed at 10 °C in the aqueous systems of pH 4 and 7. (c)  $\beta$ -Carotene microcapsules were placed at 4 °C in the aqueous systems of pH 4 and 7. Error bars represent the standard deviation of the mean of triplicate experiments. Values with different letters are significantly different ( $P < 0.05$ )

The degradation changes of  $\beta$ -carotene microencapsulated in the WPI-galactose MRPs and the WPI-galactose mixture stored at 10 °C in aqueous systems of pH 4 and 7 are shown in Fig. 5b. When  $\beta$ -carotene microcapsules were stored at 10 °C, at pH 4 and 7, the retention rate of  $\beta$ -carotene microencapsulated in the WPI-galactose MRPs was higher than that of  $\beta$ -carotene microencapsulated in the WPI-galactose mixture ( $P < 0.05$ ). Again, as the storage time increased, the retention rates of  $\beta$ -carotene microencapsulated in both the WPI-galactose MRPs and the WPI-galactose mixture significantly decreased ( $P < 0.05$ ). For instance, stored at pH 4 and 10 °C for 120 h, the retention rates of  $\beta$ -carotene microencapsulated in both the WPI-galactose MRPs and the WPI-galactose mixture decreased to 21.23% and 13.47%, respectively. And stored at pH 7 and 25 °C for 120 h, the retention rates decreased to 31.26% and 24.72%, respectively.

The degradation changes of the microcapsules when stored at 4 °C in aqueous systems of pH 4 and 7 are shown in the Fig. 5c. In these conditions, the  $\beta$ -carotene retention rate of the WPI-galactose MRPs microcapsules was higher than that of those prepared with the WPI-galactose mixture ( $P < 0.05$ ). As before, the retention rate of both significantly decreased over a longer storage period ( $P < 0.05$ ). Stored for 120 h at pH 4 and 10 °C, for example, the retention rates of  $\beta$ -carotene microencapsulated in the WPI-galactose MRPs and in the WPI-galactose mixture decreased to 29.13% and 23.32%, respectively. And over a period of 120 h stored at pH 7 and 25 °C, their retention rates also decreased to 38.89% and 32.76%, respectively.

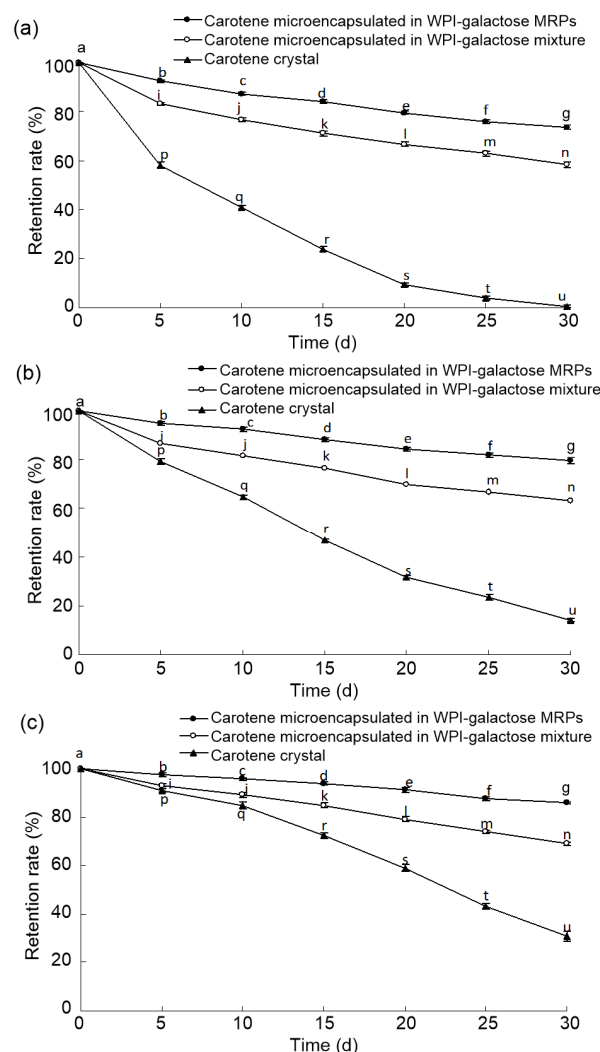
At 25, 10, and 4 °C, the retention rates of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and in the WPI-galactose mixture stored at pH 4 were lower than those in the aqueous system of pH 7. This might be due to materials forming around the microcapsules containing little ions and pro-oxidants, which could easily be adsorbed to the surface of the emulsion droplets when they were charged negatively. This would make the  $\beta$ -carotene more easily oxidized (Faraji *et al.*, 2004). In the aqueous systems of pH 4 and 7, the retention rate of  $\beta$ -carotene microencapsulated in the WPI-galactose MRPs was higher than that of  $\beta$ -carotene microencapsulated in the WPI-galactose mixture, probably due to the greater thickness and compactness of WPI-galactose MRPs. It was

also validated that glycosylated WPI enhanced  $\beta$ -carotene emulsion stability because the glycosylated wall materials provided the interface layer of the microcapsules with a greater thickness and density, which could prevent free radicals from oxidation (Al-Hakkak and Al-Hakkak, 2010).

### 3.5 Effect of air on the degradation of $\beta$ -carotene microcapsules

The effects of air on the degradation of  $\beta$ -carotene and  $\beta$ -carotene microencapsulated in both WPI-galactose MRPs and the WPI-galactose mixture at 25, 10, and 4 °C are shown in the Fig. 6. With a storage time ranging from 0 to 30 d, the retention rate of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs was remarkably higher than that of  $\beta$ -carotene microencapsulated in the WPI-galactose mixture and that of the  $\beta$ -carotene crystal at all three temperatures ( $P < 0.05$ ). The retention rates of  $\beta$ -carotene and  $\beta$ -carotene microencapsulated in both the WPI-galactose MRPs and the WPI-galactose mixture significantly decreased as storage time increased from 0 to 30 d at 25, 10, and 4 °C ( $P < 0.05$ ). For instance, when the storage time was 30 d at 25 °C, the retention rates of  $\beta$ -carotene microencapsulated in the WPI-galactose MRPs and in the WPI-galactose mixture decreased to 73.27% and 58.16%, respectively, while the  $\beta$ -carotene content of the crystals was almost wiped out. With a storage time of 30 d at 10 °C, the retention rates of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and the WPI-galactose mixture and the  $\beta$ -carotene crystals decreased to 78.96%, 63.47%, and 14.21%, respectively. At 4 °C for 30 d, these retention rates decreased to 86.12%, 69.27%, and 31.28%, respectively.

Under the action of air at 25, 10, and 4 °C, the degradation of the  $\beta$ -carotene crystals was more than that of both types of microcapsules. The  $\beta$ -carotene crystal therefore had the worst storage stability, due to its unprotected surface material and direct contact with air, where  $\beta$ -carotene could quickly be oxidized (Ribeiro and Cruz, 2005). Furthermore, the retention rate of  $\beta$ -carotene microencapsulated in the WPI-galactose mixture was higher than that of  $\beta$ -carotene crystal at 25, 10, and 4 °C. The wall materials of the microcapsules could reduce degradation of the core material. This also indicated that smaller molecules of galactose provided steric hindrance and thus prevented



**Fig. 6** Effects of air on degradation of  $\beta$ -carotene crystal and  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and WPI-galactose mixture at 25 (a), 10 (b), and 4 °C (c)

Error bars represent the standard deviation of the mean of triplicate experiments. Values with different letters are significantly different ( $P < 0.05$ )

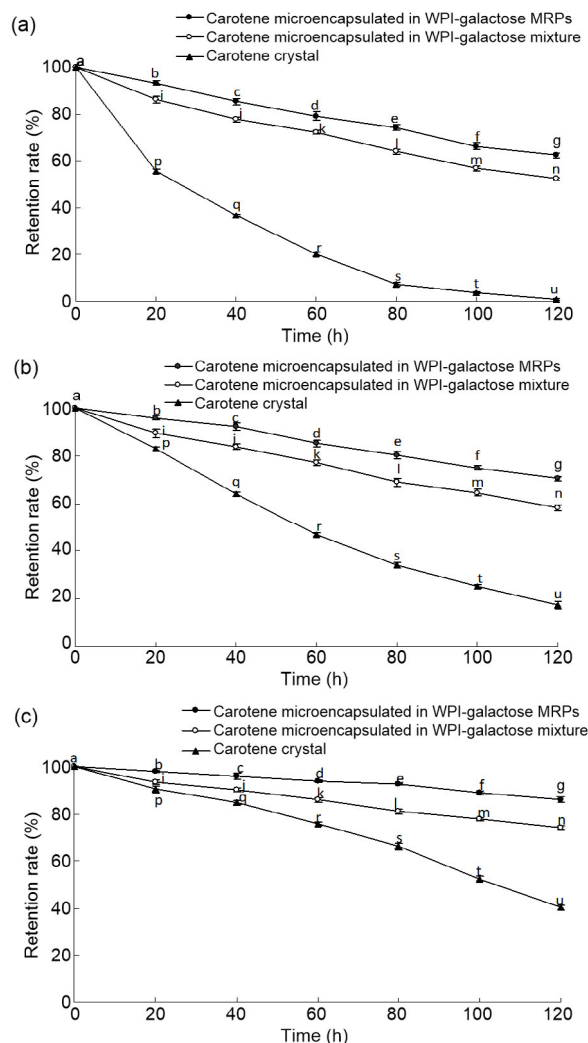
the invasion of oxidizing substances to some extent (Klinkesorn *et al.*, 2005; Chen *et al.*, 2010). Stability was strongest in the  $\beta$ -carotene microencapsulated in WPI-galactose MRPs, due to better density of the wall material and the decreased degradation of the core materials by free oxygen radicals and pro-oxidants through the interface layer of the microcapsules (Villiere *et al.*, 2005; Waraho *et al.*, 2011). The higher stability of microcapsules with wall material made from WPI-galactose MRPs may be related to MRPs' antioxidant activity (Augustin *et al.*, 2006; Drusch *et al.*, 2009).

### 3.6 Effect of incandescent light on the degradation of $\beta$ -carotene microcapsules

The effects of incandescent light on the degradation of  $\beta$ -carotene and  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and WPI-galactose mixture at 25, 10, and 4 °C are shown in Fig. 7. With storage time ranging from 0 to 120 h in the presence of incandescent light, the retention rate of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs was remarkably higher than that of  $\beta$ -carotene microencapsulated in WPI-galactose mixture and that of  $\beta$ -carotene crystal at 25, 10, and 4 °C ( $P < 0.05$ ). And the retention rates of  $\beta$ -carotene and  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and the WPI-galactose mixture significantly decreased with a storage time ranging from 0 to 120 h at 25, 10, and 4 °C in the presence of incandescent light ( $P < 0.05$ ).

For instance, at 25 °C for 120 h in the presence of incandescent light, the retention rates of  $\beta$ -carotene microencapsulated in both the WPI-galactose MRPs and the WPI-galactose mixture decreased to 60.49% and 53.16%, respectively, while that of the  $\beta$ -carotene crystals was almost wiped out. At 10 °C for 120 h in the presence of incandescent light, these retention rates decreased to 67.93%, 61.27%, and 17.38%, respectively. At 4 °C for 30 d, these retention rates again decreased to 85.47%, 74.23%, and 39.86%, respectively.

Under the action of incandescent light at 25, 10, and 4 °C, the degradation of  $\beta$ -carotene crystals was higher than that of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and that of  $\beta$ -carotene microencapsulated in the WPI-galactose mixture ( $P < 0.05$ ). Again  $\beta$ -carotene crystal had the worst storage stability, as the incandescent light led to isomerization and oxidation of unsaturated double bonds in the  $\beta$ -carotene molecule. The stability of the wall material made from WPI-galactose MRPs was stronger than those made from the WPI-galactose mixture. It could be that the WPI-galactose MRPs formed a denser microcapsule wall, providing a thicker interfacial layer than the WPI-galactose mixture (Oliver *et al.*, 2009; O'Regan and Mulvihill, 2010; Wong *et al.*, 2011). MRPs also have better antioxidation activity and prevent the diffusion of free radicals. Over the same storage duration,  $\beta$ -carotene retention rate was lowest at 25 °C and highest at 4 °C, the higher storage



**Fig. 7** Effects of incandescent light on degradation of  $\beta$ -carotene crystal and  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and WPI-galactose mixture at 25 (a), 10 (b), and 4 °C (c)

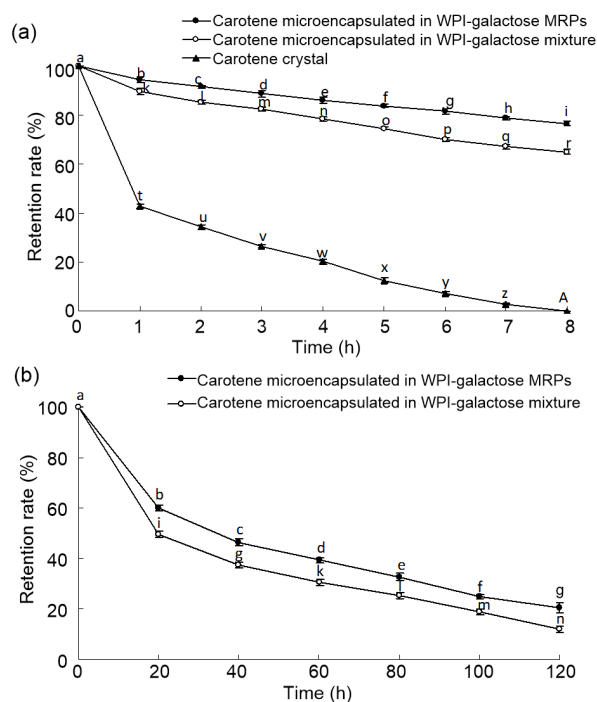
Error bars represent the standard deviation of the mean of triplicate experiments. Values with different letters are significantly different ( $P < 0.05$ )

temperature resulting in the deterioration of dense wall materials, thus reducing the retention rate of  $\beta$ -carotene.

### 3.7 Effect of UV irradiation on degradation of $\beta$ -carotene microcapsules

The effects of UV irradiation on the degradation of  $\beta$ -carotene and  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and WPI-galactose mixture stored at 25 °C for 8 and 120 h under sealed conditions are shown in Fig. 8.





**Fig. 8** Effects of UV irradiation on degradation of  $\beta$ -carotene crystal and  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and WPI-galactose mixture at different storage time

(a) Microcapsules were placed under UV irradiation for 8 h. (b) Microcapsules were placed under UV irradiation for 120 h. Error bars represent the standard deviation of the mean of triplicate experiments. Values with different letters are significantly different ( $P < 0.05$ )

During the UV irradiation process, again the retention rate of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs was remarkably higher than that of  $\beta$ -carotene microencapsulated in WPI-galactose mixture and that of  $\beta$ -carotene crystal ( $P < 0.05$ ). The retention rates of all three significantly decreased under UV irradiation as storage time increased ( $P < 0.05$ ). For instance, under UV irradiation at 25 °C for 8 h, the retention rate of  $\beta$ -carotene microencapsulated in the WPI-galactose MRPs and that of  $\beta$ -carotene microencapsulated in the WPI-galactose mixture decreased to 76.87% and 64.58%, respectively, while that of the  $\beta$ -carotene crystals was again almost wiped out. Over duration of 120 h at 25 °C under UV irradiation, the retention rates of  $\beta$ -carotene microencapsulated in WPI-galactose MRPs and the WPI-galactose mixture decreased to 18.23% and 12.47%, respectively.

Under UV irradiation for 8 h, the retention rate of  $\beta$ -carotene crystals was wiped out. The  $\beta$ -carotene

crystals came into direct contact with the UV radiation, which led to the isomerization of unsaturated double bonds in the  $\beta$ -carotene molecule and caused oxidative deterioration. In the presence of UV light, microcapsules with wall material made from the WPI-galactose MRPs had better stability than those with wall material consisting of the WPI-galactose mixture, due to the higher density of the WPI-galactose MRPs, which slowed the energy of the UV radiation.

## 4 Conclusions

$\beta$ -Carotene microcapsules were prepared using WPI-galactose MRPs as wall materials by spray drying. Their surface morphology was smooth, and was a less concave-convex sphere. The particle size ( $D_{50}$ ) of microcapsules made with WPI-galactose MRPs was smaller than that of microcapsules made with the WPI-galactose mixture. Stability studies were carried out under different storage conditions of varying temperatures, pH values, airs, and lights (incandescent and UV).  $\beta$ -Carotene microcapsules using WPI-galactose MRPs for wall materials showed good stability against temperature, pH values, airs, incandescent lights, and UV lights. The experimental results showed that the retention rates of  $\beta$ -carotene microcapsules prepared with WPI-galactose MRPs and WPI-galactose mixture as well as  $\beta$ -carotene crystal were significantly decreased as the storage time increased. Using WPI-galactose MRPs as coating materials could more effectively enhance the stability of  $\beta$ -carotene microcapsules under varying environmental conditions.

## Compliance with ethics guidelines

Zhan-mei JIANG, Li-na BAI, Nan YANG, Zhi-biao FENG, and Bo TIAN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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## 中文概要

**题目:** 研究以乳清蛋白美拉德反应产物为壁材的  $\beta$ -胡萝卜素微胶囊稳定性

**目的:** 从温度、pH、空气、白炽光照和紫外光照五个方面, 研究环境变化对微胶囊贮存稳定性的影响。

**创新点:** 首次证实乳清蛋白与半乳糖美拉德反应产物 (WPI-半乳糖 MRPs) 有助于提高  $\beta$ -胡萝卜素微胶囊的贮存稳定性。

**方法:** 利用扫描电镜观察  $\beta$ -胡萝卜素微胶囊形态特征; 用激光粒度分析微胶囊颗粒的大小; 以微胶囊的保留率为检测指标, 研究温度、pH、空气、白炽光照及其紫外光照对微胶囊贮存稳定性的影响。

**结论:** 以 WPI-半乳糖 MRPs 为壁材的  $\beta$ -胡萝卜素微胶囊表面光滑完整, 没有裂缝和孔隙, 有少量典型的凹陷; 以 WPI-半乳糖 MRPs 为壁材的微胶囊粒径比以 WPI-半乳糖混合物为壁材的微胶囊粒径小; 从温度、pH、空气、白炽光照及紫外光照五个方面进行研究发现, 以 WPI-半乳糖 MRPs 为壁材的  $\beta$ -胡萝卜素微胶囊贮存稳定性显著优于以 WPI 半乳糖的混合物为壁材的  $\beta$ -胡萝卜素微胶囊贮存稳定性; WPI-半乳糖 MRPs 有助于提高  $\beta$ -胡萝卜素微胶囊的稳定性。

**关键词:** 美拉德反应产物 (MRPs); 乳清蛋白;  $\beta$ -胡萝卜素; 微胶囊; 稳定性