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Inhibition of the double-edged effect of curcumin on Maillard reaction in a milk model system by a nanocapsule strategy

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ABSTRACT

In this work, the double-edged effect of curcumin (Cur) on Maillard reaction (MR) in model milk pasteurization process was demonstrated for the first time, where Cur inhibits MR at low concentrations (<5 μg/mL), while promotes it at high concentrations (>5 μg/mL). A nanocapsule strategy was introduced to resolve the disadvantages of Cur on MR at high temperature. In vitro experiments confirmed that curcumin @ polyvinylpyrrolidone nanocapsules (Cur@PVP NCs) synthesized by a one-pot method can control the continuous and slow release of Cur and exert inhibitory effect on MR by competing with lactose for casein, trapping the reactive dicarbonyl compounds and scavenging ROS, which might provide a new strategy for the application of functional food ingredients in the control of MR during food heat processing, including milk pasteurization.

1. Introduction

Maillard reaction (MR), also known as nonezymatic browning, can be considered as one of the most important chemical reactions occurring in the process of food processing, particular in heating. It is formed by the condensation between ε and α-amino groups of amino acids and carbonyl groups of reducing sugars and the subsequent numerous reactions. The resultant compounds during this reaction are called Maillard reaction products (MRP) (Silvan, Assar, Srey, Castillo, & Ames, 2011). Apart from improving food quality attributes such as color, flavor and taste, MR also induces some unwanted effect related to pathological consequences in vivo. It has been reported that the amount of MRP in plasma and tissues is associated with biological disorder like diabetic complications, including neuropathy, nephropathy, retinopathy, Alzheimer's disease and so on (Tessier & Birlouez-Aragon, 2012). In addition, considerable evidence has also stated that the advanced stage products of MR binding to the RAGE activates the proinflammatory transcription factor nuclear factor-kappa B and results in immortal cell activation and celluar dysfunction (Bierhaus et al., 2005; Lu et al., 2010; Wu et al., 2015). Hence, it is vital to control the MR in food heat processing.

Curcumin (Cur), natural polyphenolic compound, is isolated from the rhizome of turmeric and has been used as a spice and food coloring in Asia cooking for several decades (Khopde, Priyadarsini, Venkatesan, & Rao, 1999; Liu et al., 2016; Sahu, Kasoju, & Bora, 2008). In recent years, Cur has been applied in the field against MR due to its excellent antioxidant activity. For example, Sajithlal and co-workers have reported that the level of nonenzymic antioxidants maintains normal in diabetic rats by Cur administration, which indicates that Cur is able to effectively suppress MR (Sajithlal, Chithra, & Chandrakasan, 1998). Moreover, the inhibitory effect of Cur on the formation of mutagenic MRP in an in vitro model system has been reported (Kolpe, Ramaswamy, Rao, & Nagabhushan, 2002). However, these experiments were generally carried out in physiological conditions, which could not exactly reflect the real effect of Cur on MR because MR is easier to take place at high temperature. Therefore, to study the effect of Cur on MR at high temperature possesses a practical significance.

Milk is a kind of daily necessity for most people and its nutritional value is high. However, heat treatment during the process of sterilization may lead to an obvious decrease of the milk nutritional value, as it reduced the protein functional properties, diminishes the bioavailability of the essential amino acids, eventually affects food disgestibility and determines a potential increase of specific

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allerge (Renzone, Arena, & Scaloni, 2015). In this work, we synthesized Cur@PVP NPs by a one-pot method and investigated their anti-glycation effect on MR in casein/lactose system at 60 °C, which simulated milk pasteurization process. By measuring the amount of various intermediate products of MR, we could conclude the mechanism of Cur and Cur@PVP NPs on MR. Furthermore, we also verified this mechanism by characterizing carbonyl content and the secondary structures. The mechanism could provide a reference for further investigation about MR, meanwhile, open an avenue to develop application of traditional food functional component by the nanotechnology.

2. Materials and methods

2.1. Reagents

Casein, 4-dimethylaminopyridine (DMAP), nitroblue-tetrazolium (NBT), Girard-T reagent, ortho-phthalaldehyde (OPA), Tween-80, and 2,4-dinitrophenylhydrazine (DNPH) were purchased from Sigma (St. Louis, USA). Cur and PVP were purchased from Aladdin industrial Corporation (Shanghai, China). Triethylamine (TEA) and dimethyl sulfoxide (DMSO) were purchased from Kelong Chemical Reagent Factory (Chengdu, China). Alpha-lactose was purchased from Sinopharm Group Chemical Reagent Co.Ltd (Shanghai, China). All reagents were used without further purfication and ultrapure water was used in all experiments.

2.2. Preparation of Cur@PVP NCs

Cur@PVP NCs were synthesized by the one-pot method according to a previous report with some modification (Yu et al., 2014). Typically, 0.9 g of PVP, 0.3 g of DMAP, 0.6 mL of TEA and 30 mg of Cur were added into DMSO (30 mL) and stirred in the dark at room temperature with the protection of N_2. After 24 h, unbound entities in the mixture solution were completely removed by dialysis for 3 days. Then, Cur@PVP NCs were lyophilized and kept in the lucifuge condition at 4 °C.

2.3. Physical characterization

The morphology and particle size of Cur@PVP NCs were characterized by scanning electron microscope (SEM, s-4800, Hitachi High-Tech). UV-vis spectra and FT-IR spectra obtained by an ultraviolet-visible spectrophotometer (UV-2550, Shimadzu) and a Fourier transform infrared spectroscopy (FT-IR, TENSOR27, Bruker), respectively, were used to investigate the possible chemical interactions between Cur and PVP.

2.4. Drug loading capability and drug loading efficiency

The method of measuring the drug loading capability (DLC) and drug loading efficiency (DLE) of Cur was based on a standard curve method (Zheng, Liu, Guan, & Xie, 2015). Firstly of all, different amount of free Cur was dissolved in DMSO solution (80 mL/100 mL) and the absorbance at 427 nm (Abs_{427}) was measured using a UV-vis spectrophotometer to establish a standard curve. Then a certain amount of Cur@PVP NPs solution was carried out under the identical condition and analyzed the concentration of Cur according to the below equation, respectively:

$$\text{DLC}\ (\%) = (\text{weight of loaded Cur} / \text{weight of nanocapsules}) \times 100\% \quad (1)$$

$$\text{DLE}\ (\%) = (\text{weight of loaded Cur} / \text{initial weight of Cur}) \times 100\% \quad (2)$$

2.5. In vitro release and stability of Cur@PVP NCs

In vitro cumulative release of Cur from Cur@PVP NCs were performed using a dialysis bag diffusion technique (Gao et al., 2013), which has been described in Supplementary Material.

The stability of Cur@PVP NCs was evaluated at room temperature and 60 °C, respectively. Free Cur solution was carried out as a contrast. At selected time intervals, 0.5 mL solution was removed to measure Abs_{427}. The collected data were expressed as absorbance changing with time and used to analyze the stability of Cur@PVP NCs.

2.6. Preparation of model milk system

Because of the complexity of milk, we employed casein/lactose system to evaluate the effect of Cur and Cur@PVP NCs on MR at high temperature. Briefly, casein and lactose were dissolved in phosphate buffered solution (PBS) (0.2 mol/L, pH 6.7). Then 1 mL of casein solution was mixed with 1 mL of lactose solution and different concentration of Cur solution. After diluting to 3 mL with PBS, the final concentration of casein and lactose were 24 mg/mL and 50 mg/mL, respectively. The final concentration of Cur in different systems separately reached to 1 μg/mL, 5 μg/mL, 10 μg/mL, 15 μg/mL and 20 μg/mL. Finally, the mixture solution was equably oscillated and incubated at 60 °C for 20 h to obtain enough MRP for detection.

2.7. Exploration of the effect and the mechanism of Cur and Cur on MR

Firstly, the effect of Cur and Cur@PVP NCs on MR was evaluated by the fluorescence intensities. Briefly, the glycated samples (800 μL) were diluted to 2.4 mL and the fluorescence intensities were measured via a fluorescence spectrophotometer (F-4600, Hitachi High-Tech) at the excitation wavelength of 370 nm. The collected data were calculated according to the following equation and expressed in the form of the percent inhibition:

$$\text{Inhibitory rate}\ (\%) = (F_2 - F_1) / (F_2 - F_3) \times 100 \quad (3)$$

where F_1 is the fluorescence intensity of Cur or Cur@PVP NCs treatment group (casein/lactose/Cur or Cur@PVP NCs); F_2 is the fluorescence intensity of positive control group (casein/lactose/PBS); F_3 is the fluorescence intensity of negative control group (casein/PBS).

Then we explored the mechanism by measuring the amount of various intermediate products. Detailed information was as follow:

Fructosamine, as the typical Amadori products in the early stage of MR, was detected the spectrophotometry method based on the reaction with NBT reagent (Danese, Montagnana, Nouvenne, & Lippi, 2015). The sample solution (25 μL) was incubated with 100 μL of NBT reagent (0.5 mmol/L) in sodium carbonate buffer at 37 °C for 15 min. After incubation, all samples were determined Abs_{530} and all collected data were expressed as absorbance changing with time.

Glyoxal, a kind of typical 1,2-dicarbonyls, is formed by further degradation of Amadori products or oxidation of Schiff bases in the medium stage of MR. It was measured by Girard's reagent T. Firstly, 200 μL of glycated sample was added into the mixture containing 50 μL of Girard-T stock solution and 850 μL of sodium formate. Then the above mixture was incubated at room temperature for 1 h. Absorbance was recorded over a wavelength range of 260–350 nm using a UV-vis spectrophotometer. The maximum absorbance at