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Simulated digestive behavior of naringin microspheres and its influence on yogurt: The rheology and antioxidant activities

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Abstract In this study, naringin was encapsulated in microspheres and its simulated digestive behavior *in vitro* was examined. Then naringin microspheres was added in yogurt to investigate the rheology and antioxidant activities. The results indicated that encapsulating naringin in microspheres delayed its digestion in the stomach, allowing more release in the intestinal part. All kinds of yogurt were solid-like in nature and the addition of microspheres increased the elastic modulus and viscosity. The naringin and microspheres incorporation enhanced the total phenolic content of the yogurt to 6.7 and 8.8 mg of gallic acid equivalent/mL, respectively. All kinds of yogurt demonstrated more than 80% scavenging ability for hydroxyl radicals at 20 μL whey/mL. The addition of microspheres improved the DPPH radical scavenging ability of yogurt. This study provides a new idea for the application of polyphenols in food and the development of functional yogurt.

Keywords: naringin; simulated digestion; yogurt; rheology; antioxidant activity

1 Introduction

Naringin is a polyphenol rich in Citrus fruits as the main bitter component [1]. It is also one of the active ingredients in Rhizoma Drynariae, which is a white to yellowish crystalline powder with antioxidant, anti-inflammatory, and intestinal health-improving properties [2-6].

Microspheres is a kind of dosage form in which functional ingredients are encapsulated through wall material for the purpose of avoiding environmental exposure, masking undesirable flavors, and increasing solubility and stability [7]. Through microsphere technology, it is also possible to improve the antimicrobial and antioxidant activity, and develop the potential for the application of biologically active ingredients in food [8, 9]. Yogurt is a fermented dairy product made of raw milk or milk powder, obtained by fermentation. Yogurt is becoming increasingly popular as a source of energy and nutrition due to its unique flavor and health benefits [10]. Currently, there are some reports of functional ingredients added to yogurt through microsphere process [11, 12].

In this study, the release behavior of naringin microspheres was investigated during simulated digestion *in vitro*. The microspheres were then incorporated into yogurt to investigate its influence on the rheology and antioxidant activities.

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These authors have no conflict of interest to declare.

2 Materials and methods

2.1 Materials and reagents

Naringin was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China); Ethyl oleate was obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China); Skimmed milk powder was purchased from Shandong Yibaolai Biotechnology Co., Ltd (Shandong, China); Chicory fructooligosaccharides were bought from Shanxi Fulbon Biotechnology Co., Ltd. (Shanxi, China); Pectin and aspartame were obtained from Ji'nan Jieyang Biotechnology Co., Ltd. (Shandong, China). All other chemicals and reagents were of analytical grade.

2.2 Appearance and microstructure

Naringin microspheres were prepared according to the previous study [13]. An appropriate number of microspheres was dispersed on a microscope slide and observed through an optical microscope (DMBA450, Motic China Group Co., Ltd., China).

2.3 In vitro simulated digestion study

Naringin microspheres were weighed and added to 200 mL simulated gastric fluid with stirring at 37 ºC. The simulated gastric fluid was collected at predetermined time intervals, and an equal volume of fresh media was immediately replaced. After 120 min gastric digestion, the microspheres were separated by filtration, then mixed into 200 mL of simulated intestinal fluid, and the samples were taken at the same time intervals and operation.

The obtained release results were fitted to kinetic models including zero-order, first-order, Higuchi and Ritger-Peppas matrix. The relevant correlation coefficients were taken into consideration to select the best model.

2.4 Rheological measurements of yogurt

Yogurt preparation was based on our previous study [13]. The viscoelastic properties of yogurt were analyzed by a rotational rheometer (AR 2000ex, TA Instruments, USA) with a 40 mm conical plate geometry [14]. The relationship between elastic modulus (G') and viscous modulus (G") and frequency (0.1−10 Hz) was obtained by placing 2 mL yogurt directly under the conical plate, and performing dynamic frequency scanning at a strain of 0.5%. The apparent viscosity was determined at a shear rate of 0−100 s^{-1}.

2.5 Determination of total phenols content

The 10 mL of yogurt was mixed with 10 mL of methanol containing 0.5% concentrated hydrochloric acid in a centrifuge tube, homogenized for 2 min, and centrifuged at 4 000 r/min for 5 min. The samples were placed at −20 ºC for 1 h to fully precipitate the proteins, and then centrifuged again to obtain the supernatant. The total phenols content was determined by the method described by Wang, et al. [15]. The results were expressed as mg of gallic acid equivalent/mL of sample (GA eq/mL).

2.6 Antioxidant activity

The yogurt was centrifuged at 4 000 r/min for 15 min to obtain the supernatant. Appropriate amount of supernatant was pipetted and diluted with 80% methanol or water to obtain whey solutions of different concentrations. The DPPH and hydroxyl radical scavenging abilities were determined by DPPH and hydroxyl radical Reagent Kit Product (Nanjing Jiancheng Bioengineering Institute, China) [16].

3 Results and discussion

3.1 Optical microscope analysis

As shown in Fig. 1, naringin microspheres were round in shape, non-adherent to each other, and uniform in size. Huang, et al. [17] fabricated lutein microspheres by cross-linking sodium alginate with Ca^{2+}, which were found with a rough surface, small pores and spherical shape by scanning electron microscopy images. Ruan, et al. [18] prepared tea polyphenol-collagen-alginate microspheres, and the similar appearance and morphology was observed.

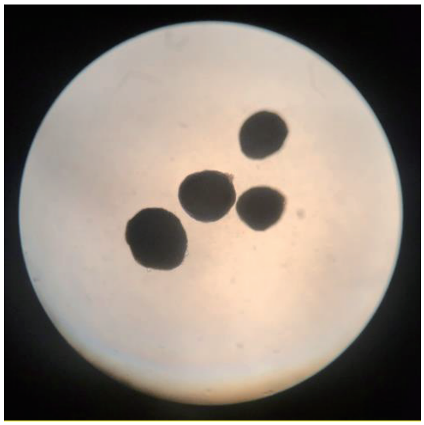

Fig. 1 Optical microscope pictures of naringin microspheres (10 × 40)

3.2 In vitro simulated digestion study

As shown in Fig. 2, the release rates of naringin from the microspheres under simulated gastrointestinal conditions were all characterized by a rapid increase followed by a gradual slowing down to a plateau. The microspheres released about 20.3% of naringin during the first 30 min in gastric fluid, and then proceeded to a slow-release stage, releasing about 27.3% at the end of the gastric digestion phase. It was due to the partial protonation of the carboxyl groups in calcium alginate under highly acidic conditions, which caused the pores in the microspheres become narrow, restricting the diffusion of digestive enzymes into the interior, and also inhibiting the release of naringin [17].

At 20 min of the intestinal digestion phase, the release of naringin increased rapidly to 58.9%. The intestinal fluid pH exceeded the pKa value of the carboxyl groups on alginate chains, and the negative charge on the alginate molecules increased, resulting in an additional electrostatic repulsion between the alginate chains. And in the presence of trypsin, calcium alginate was hydrolyzed, causing further structural disruption of the microspheres. At the end of the intestinal digestion phase, naringin was released 72.8% from the microspheres. The above results suggested that encapsulation of naringin in microspheres could delay its digestion in the stomach and allow more digestion to occur in the intestinal phase. Since naringin can interact with the microorganism in the intestinal tract, the naringin microspheres developed in this study could be used in the foods to maintain intestinal health [19].

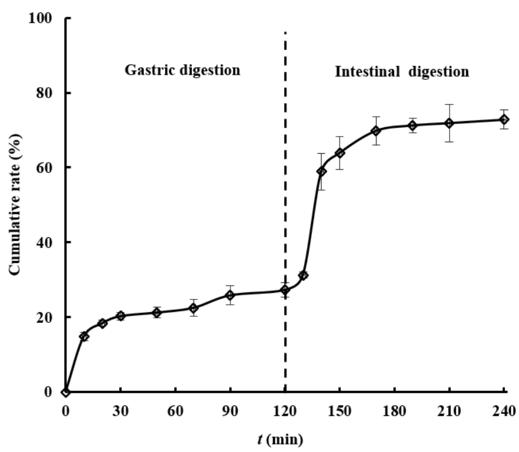

Fig. 2 The release behavior of naringin from microspheres under *in vitro* digestion conditions

The fitting results of the release behavior of naringin microspheres during simulated digestion are shown in Table 1. In the gastric digestion phase, the release behavior of naringin was more in line with the Ritger-Peppas equation (R^2 = 0.992), with an n-value less than 0.45, indicating that the release behavior at this stage was Fick diffusion [20]. While in the intestinal digestion phase, it was more consistent with the first-order kinetic equation (R^2 = 0.989).

Table 1 Model simulated for the release profiles of naringin from microspheres

Model	Gastric digestion		Intestinal digestion	
	Equation	R^2	Equation	R^2
Zero order	$M_t = 0.17\,k + 10.73$	0.598	$M_t = 0.29\,k + 20.71$	0.469
First order	$M_t = 24.29\,(1 - e^{-0.07\,k})$	0.938	$Mt = 46.18\,(1 - e^{-0.69\,k})$	0.989
Higuchi	$M_t = 2.24\,k^{1/2} + 5.06$	0.864	$M_t = 4.16\,k^{1/2} + 9.51$	0.774
Ritger-Peppas	$M_t = 8.92\,k^{0.23}$	0.992	$M_t = 16.75\,k^{0.23}$	0.918

3.3 Rheological properties

The rheological results of yogurt are shown in Fig. 3. In the range of 0.1-10 Hz, the G’ of all kinds of yogurt were higher than G”, indicating that yogurt exhibited a solid-like behavior with a weak gel structure (Fig. 3A) [21]. When the frequency was 10 Hz, the G’ of control yogurt (CY), naringin (NY) and microsphere yogurt (MY) were 124.8, 101.9 and 172.5 Pa, thus the stability of the yogurt was hypothesized to be MY > CY > NY. The addition of microspheres enhanced the G’ of yogurt owing to the silk fibroin aggregate and connect the casein proteins in the yogurt to form a tight structure, and the water-holding property of the microspheres also improved the stability of the yogurt. The ratio of G” to G’ is tanδ, which exhibits liquid-like properties when tanδ ≥ 1, and the opposite has solid-like properties [22]. All kinds of yogurt showed tan$\delta \leq 1$ (0.3 < tanδ < 0.4) in the selected frequency range, demonstrating that they were all solid-like in nature and the lower tanδ represents a firmer yogurt [23].

As shown in Fig. 3B, the viscosity of yogurt decreased rapidly with increasing shear rate, exhibiting shear-thinning behavior. Gonçalves et al. encapsulated curcumin in solid lipid nanoparticles and incorporated it into yogurt, also observed a shear thinning behavior [24]. The reason was the microstructure of the yogurt was disrupted under the shear force. Initially, the viscosities were 15.2 (CY), 14.8 (NY) and 21.5 Pa (MY), showing the addition of microspheres increased the viscosity values of the yogurt, which was similar to the measurements of G’ and G’’ described above.

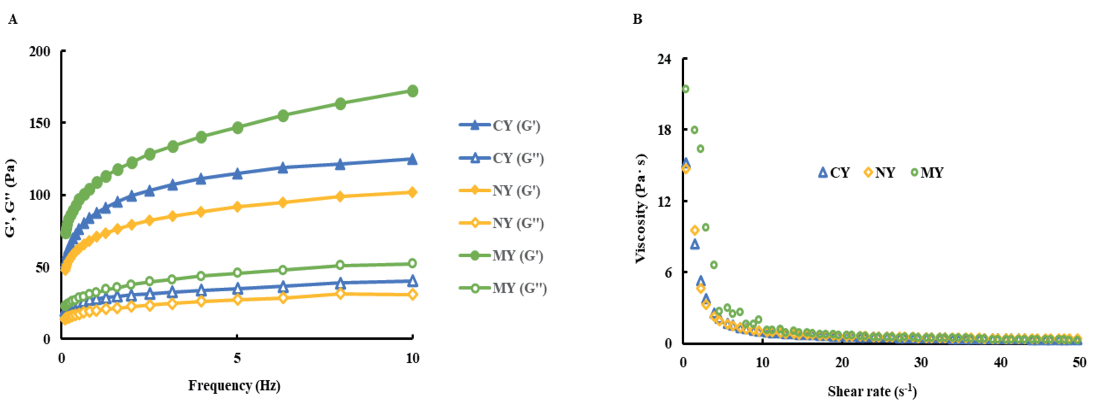

Fig. 3 The effect of frequency on viscoelasticity (A) and the effect of shear rate on apparent viscosity (B)

3.4 Total phenolic contents

The total phenolic contents in CY, NY and MY were determined and calculated to be 0.9 ± 0.1, 6.7 ± 0.2 and 8.8 ± 0.1 GA eq/mL, respectively. The phenolic compounds in CY were considered to be the presence of milk powder and chicory oligofructose with a small number of phenolic compounds. According to our previous study results, the solubility of naringin in water was enhanced from 0.85 mg/mL to 1.01 mg/mL after encapsulation, due to the conversion of naringin from the crystalline to amorphous state [13]. The more total phenolic content in MY was mainly attributed to the presence of more naringin in the whey due to the increased solubility of naringin, and also the possible presence of phenolic compounds in the excipients of the microspheres, such as sodium alginate. Sodium alginate is extracted from brown algae, and Liu et al. detected polyphenolic substances in it using the fluorescence method [25]. It was deduced that NY and MY may have better antioxidant activity due to the antioxidant capacity possessed by phenolic compounds.

3.5 Antioxidant activity

Fig. 4 shows the antioxidant activity of yogurt. The concentration of whey was positively correlated with its scavenging ability for DPPH radicals in the selected concentration ranges (Fig. 4A). In comparison to CY, both NY and MY displayed higher scavenging capacity at all concentrations, which was due to the higher total phenolic content. All kinds of yogurt samples showed effective scavenging of hydroxyl radicals (Fig. 4B). Ruan et al. also found that cranberry fruit powder fortified yogurt had a better scavenging ability for hydroxyl radicals than DPPH [26]. Some bioactive peptides contained in yogurt could express strong antioxidant capacity [27]. At the concentration of 20.0 μL whey/mL, the hydroxyl radicals scavenging ability tended to be consistent and equal to that of Vitamin C (VC), suggesting that all kinds of yogurt had favorable hydroxyl radicals scavenging ability.

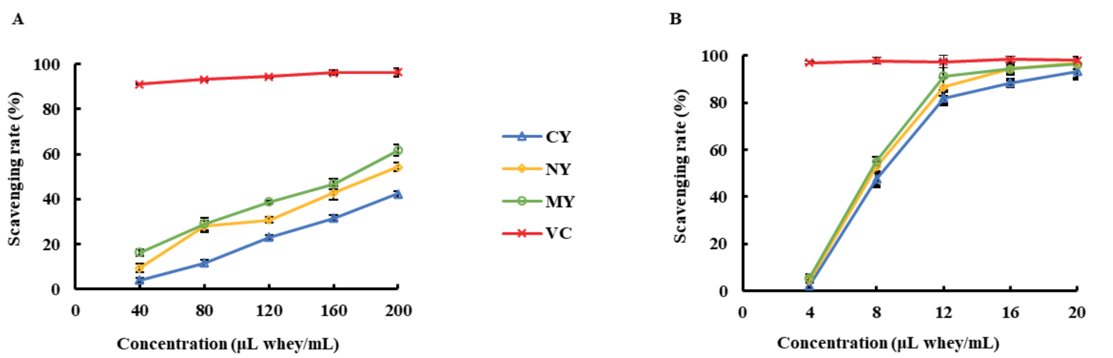

Fig. 4 DPPH (A) and hydroxyl (B) radical scavenging activity of VC, and the whey of CY, NY and MY

4 Conclusion

The current study determined the simulated digestive release of naringin microspheres and its effect on yogurt rheology and antioxidants. Microspheres delayed naringin digestion in the simulated stomach, allowing more digestion to occur in the intestinal phase. The release of naringin was in accordance with the Ritger-Peppas matrix in the gastric stage, while with the first-order kinetic matrix in the intestinal part. The microspheres increased the elastic modulus and viscosity of the yogurt and enhanced the total phenolic content to 8.8 GA eq/mL. All kinds of yogurt exhibited good scavenging activity of DPPH radicals, and the scavenging ability for hydroxyl radicals tended to be consistent with that of the VC at 20.0 μL whey/mL. This study provides a reference for the development and utilization of functional yogurt.

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