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The effects of carrageenan on stability of arachin and the interactions between them

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ABSTRACT

The influence of k-carrageenan on the stability of arachin was analyzed. K-carrageenan could significantly enhance the stability of arachin solution. 0.04% k-carrageenan was the most effective when the concentration of arachin was 2.0%. The pH value, ionic strength and temperature significantly affected the stability of mixture system. The system showed good stability at pH 7.0. High temperature was not beneficial for the system. Na^+ and Ca^{2+}, especially Ca^{2+}, could induce phase separation of the system at low concentration. The interactions between arachin and k-carrageenan were studied using fluorescence spectroscopy, DSC, SEM and SDS-PAGE analyses. The results revealed that k-carrageenan could adsorb to the surface of arachin. The adsorption may modify the tertiary structure of arachin and the polarity of microenvironment decreased. The complex showed a comparatively homogeneous network structure from SEM analysis. In addition, the thermal stability of arachin was also enhanced by addition of k-carrageenan. T_d and ΔH increased significantly, whereas a significant decrease was determined in ΔT_d.

1. Introduction

Peanut is an important crop grown in China and worldwide. China is one of the largest producers of peanut and has the largest yield in the world (Liu, Zhao, Zhao, Ren, & Yang, 2012). Peanut is a good source of protein, which contains about 25–32% protein. Moreover, peanut protein is considered as good nutritional quality for its high essential amino acid content. The major proteins of peanuts are arachin (l4 S) and conarachin I (7.8 S) and conarachin II (2 S), which account for 75–80% of the total proteins (Wu, Wang, Ma, & Ren, 2009). Among them, arachin is the major protein and easy to extract in aqueous solvents. Arachin has low thermal stability and this directly affects the application of peanut protein in food industry. The stability is the key problem in preparation and storage of peanut protein beverage. Recently, more and more researches focused on the thermal properties and functional modification of arachin (Colombo, Ribotta, & Leon, 2010; Liu, Teng, et al., 2011; Liu, Zhao, et al., 2011). Govindaraju and Srinivas (2006) reported that hydrolysis by protease could improve the emulsifying and foaming properties of purified arachin. Liu et al. (2012) used maillard reaction to improve the foaming and stability properties of peanut protein.

Prevention of phase separation or particle sedimentation is important for food production. Carrageenans, obtained from red seaweeds, are water soluble sulfated anionic galactans. They are one of the most important polysaccharides and have a long history use as gelling agents, thickeners and stabilizers in food industry (Alexander & Dalgleish, 2007). Three generic carrageenan families (kappa, iota or lambda) are commonly used. Among them, k-carrageenan is the most important commercial forms of carrageenans (Olivares, Passeggi, Ferrón, Zorrilla, & Rubiolo, 2010). It is used in many food formulations. For example, Spagnuolo, Dalgleish, Goff, and Morris (2005) found that both k-carrageenan adsorption to casein micelle surfaces and k-carrageenan helix aggregation are necessary for preventing casein micelle phase separation. The interactions between k-carrageenan and protein have been suggested to occur via localized electrostatic attractions (Alexander & Dalgleish, 2007). In addition, other factors such as K^+, Ca^{2+} and Na^+ ions, temperature and pH can also affect the stability of the mixture system (Trckova, Stetina, & Kansky, 2004).

The objectives of this work were: (i) to examine the effect of k-carrageenan concentration, pH, ionic strength and temperature on the stability of arachin/k-carrageenan system; (ii) to investigate the interactions between carrageenan and arachin and the mechanism of this process.

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2. Materials and methods

2.1. Preparation of arachin from defatted peanut flour

Defatted peanut flour was purchased from Tianshen Peanut Company (Shandong, China). Arachin was obtained from defatted peanut flour according to the report by Monteiro and Prakash (1994a). Defatted peanut flour was dispersed in 0.05 M phosphate buffer (pH 7.9, 1:20 w/v) and stirred at 25 °C for 1 h, then centrifuged at 8000 *g* for 30 min. After removing the precipitate, ammonium sulfate was added into the supernatant (24%, w/v). The mixture was kept at 4 °C for 3 h and then centrifuged. The obtained precipitate was dissolved in minimum volume of extraction buffer and dialyzed extensively against distilled water. Arachin powder was obtained by freeze-drying.

2.2. Arachin and k-carrageenan mixtures

The k-carrageenan was purchased from Wenchang Company (Hainan, China). K-carrageenan powder (0.2 g/100 mL) was dissolved in distilled water and then heated in water bath at 70 °C for 40 min with continuous magnetic stirring. Solutions of k-carrageenan (0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, and 0.18 g/100 mL) were prepared subsequently.

Arachin solution (4.0 g/100 mL) was prepared by dissolving arachin powder in sterile water with continuous stirring for 60 min at room temperature.

All solutions were kept at least for 4 h to ensure a complete hydration. Same volumes of arachin and k-carrageenan solutions were mixed together. The final concentration of arachin was 2.0% (w/v).

2.3. The effect of k-carrageenan concentration, pH, ionic strength and temperature on the stability of mixture system

2.3.1. Analytical methods

In this study, precipitation rate, viscosity, digital imagery and Zeta-potential analysis were used to evaluate the stability of the mixture system. The details were as follows:

Some amount of solution was separated by centrifugation at 5000 *g* for 15 min. Before and after removing the supernatant, the tube was weighed. The precipitation rate (R) was calculated by:

$$R\ (\%) = \frac{W_2 - W_0}{W_1 - W_0} \times 100\%$$

where W_0 was the weight of centrifuge tube (empty); W_1 and W_2 were the weights of centrifuge tube before and after removing the supernatant.

The rheological measurements were conducted on a controlled stress rheometer (C-LT0801QC, Zhonglei, Shanghai, China). All the rheological measurements were performed at 25 °C, the same temperature used for preparation of arachin from defatted peanut flour.

Images of the mixtures and separated phases were obtained by a digital camera. The images were used to observe the appearance features of the mixtures. After storage at 4 °C for 7 days, tubes contained the mixtures were photographed.

Zeta potential measurements were performed on a Zeta potential instrument (Zetasizer nano ZS90, Malvern Instruments Ltd., Britain). All the measurements were conducted at 25 °C.

2.3.2. Samples analysis

To determine the effect of k-carrageenan concentration on the stability of mixture system, mixtures containing k-carrageenan (0.00, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09and 0.10 g/100 mL) were prepared. Samples were taken on the 1st, 3rd, 7th day, respectively, and then analyzed according to the methods above.

The effect of pH on the stability was performed by adjusting the pH of the mixtures to 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 with 1.0 M HCl or 1.0 M NaOH. The mixtures contained 0.04% k-carrageenan. After storage at 20 °C for 20 h, analyses were carried out.

The influence of ionic strength was conducted by using NaCl and $CaCl_2$. The concentrations were 0.01, 0.02, 0.03, and 0.04 mol/L.

To investigate the effect of temperature on the stability of mixture system, mixtures were incubated for 20 h at 10 °C, 20 °C, 30 °C, 50 °C and 80 °C, respectively. Then samples were analyzed.

2.4. The interaction between arachin and k-carrageenan

In this experiment, the mixture system contained 2.0% arachin and 0.04% k-carrageenan. The pH value was adjusted to 7.0. Arachin solution (2.0%) without adding k-carrageenan was used as control. The details were as follows:

2.4.1. Fluorescence spectroscopy

Tryptophan (Trp) is usually used as intrinsic fluorescence to investigate protein structural changes. Samples were diluted 10 times with distilled water. After storage for 1 h, a spectrofluorophotometer (F-4500, Hitachi, Japan) was employed to analyze. The excitation wavelength was set at 290 nm. Excitation and emission slits were 5 nm. The emission was detected between 290 and 400 nm. Measurements were performed at 25 °C.

In this study, 1-anilino-8-naphthalenesulfonate (ANS) was also used to evaluate the protein structural changes. Excitation was at 390 nm and emission was collected between 400 and 600 nm. Measurements were conducted at 25 °C.

2.4.2. Differential scanning calorimetry (DSC)

Samples were obtained by freeze-dried from solution samples. The thermal properties of samples were monitored by a calorimeter (NANO DSC, TA Instruments, USA). Briefly, 0.75 g sample was put into a stainless steel cell. The scanning rate was 5.0 °C/min from 20 to 110 °C. At the same time, an empty steel cell was performed as control.

2.4.3. Scanning electron microscopy (SEM)

Powder samples from Section 2.4.2. were used to analyze. A SEM (S-570, Hitachi, Japan) was used for imaging the samples.

2.4.4. SDS-PAGE analysis

Arachin (2%) and mixtures of arachin (2%)/k-carrageenan (0.04%) solutions were treated with papain (5 mg/ml, Sigma) at 37 °C for 12 h. Samples were collected after incubation for 0, 4, 8and 12 h, and analyzed by SDS-PAGE.

SDS–PAGE electrophoresis was carried out using 5% stacking gel and 13% separating gel at 200 V. Sample (10 μL) was applied to each well. After electrophoresis, the gel was stained with coomassie brilliant blue R-250 for 1 h and discolored overnight.

2.4.5. Effect of hemicellulase on arachin-k-carrageenan system

Mixture system was treated by hemicellulase (5 mg/ml, Sigma) at 37 °C for 200 min. Samples were collected after incubation for 0, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 min, and analyzed by Zeta potential instrument.