Food Chemistry 242 (2018) 83–90

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Targeted regulation of hygroscopicity of soybean antioxidant pentapeptide powder by zinc ions binding to the moisture absorption sites

Peiyu Xue[a], Na Sun[a], Yong Li[a], Sheng Cheng[b], Songyi Lin[a,*]

[a] National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, PR China
[b] Analysis and Test Center, Dalian Polytechnic University, Dalian 116034, PR China

ARTICLE INFO

Keywords:
Soybean antioxidant pentapeptide (SAP)
Zinc chelation
Hygroscopicity
Targeted regulation

ABSTRACT

In the present study, a targeted regulation of hygroscopicity of soybean antioxidant pentapeptide (SAP) powder was explored by zinc ions binding to its moisture absorption sites. Scanning electron microscopy, X-ray diffraction analysis, Fourier transform infrared spectroscopy and an energy-dispersive X-ray spectroscope were used to confirm the formation of the SAP-zinc complex. The results showed that morphology of SAP-zinc complex belonged to crystalline nanoparticles. The moisture sorption/desorption kinetics of the SAP-zinc complex changed compared to that of the SAP. In particular, the moisture sorption capacity of the SAP decreased and the distribution of adsorbed water changed after zinc chelation. Based on the binding of zinc ions to the moisture absorption sites, the hygroscopicity of SAP powder could be target regulated. Thus, this study could provide a new method to regulate the hygroscopicity of peptide powder.

1. Introduction

Bioactive peptides containing 3–20 amino acid residues are specific protein fragments (Korhonen & Pihlanto, 2003). Based on their structural properties, amino acid composition and sequences, bioactive peptides have a positive impact on body functions and possess various functional properties, such as antioxidant, mineral binding, immunomodulatory, antimicrobial and antihypertensive functions (Cross, Huq, Palamara, Perich, & Reynolds, 2005; Gauthier, Pouliot, & Saint-Sauveur, 2006; Jia et al., 2010; McCann et al., 2006; Xu et al., 2006). Therefore, bioactive peptides could play an important role in the development of novel functional foods. Bioactive peptides prepared by spray drying or lyophilization are mainly presented in the form of peptide powders. Owing to the loose powder form and interactions with water molecules via hydrogen bonding, the peptide powders have different moisture absorption abilities (Lin, Yang, Li, Chen, & Zhang, 2016b). Hermansson (1977) studied sorption isotherms of proteins of different origins including soy protein isolate, sodium caseinate and whey protein concentrate. Moreover, the interaction between dehydrated human serum albumin and water has also been investigated (Sirotkin & Faizullin, 2004). Recently, Yang et al. (2016) studied water dynamics in egg white peptide powder, Asp-His-Thr-Lys-Glu, monitored by DVS and LF-NMR. In addition, soybean antioxidant peptide fractions with three different molecular weights were investigated to elucidate the characteristics of water mobility and distribution (Lin et al., 2016b). This hygroscopicity of peptide powders can lead to inappropriate changes of peptide powder quality, including bridging, agglomeration, compaction and even liquefaction, resulting in loss of functionality and lowered quality (Aguilera, del Valle, & Karel, 1995). Therefore, these inappropriate changes could complicate their direct utilization in food processing.

Moisture absorption capacity of protein materials depends primarily on the number and availability of the two types of hydrophilic groups, including the polar side chains, the carboxyl and imido groups of the peptide bonds, which are capable of binding water molecules through hydrogen bond formation (Leeder & Watt, 1974). Speakman (1944) postulated that water sorption by proteins occurred onto polar side chains at low humidities, spread to peptide linkages and then to multilayer formation at higher humidities. In addition, it has been reported that water sorption of polypeptides depended on the reactivity of polar groups on the side chains (Guillet, Seytre, May, & Vallet, 1975). In particular with poly-L-alanine, water is absorbed through both a hydrogen bonding mechanism and non-bonded dipole–dipole forces (Baddiel, Breuer, & Stephens, 1972).

Bioactive peptides, which are composed of His, Ser Cys, Glu, and Asp, have different degrees of hygroscopicity due to the presence of polar side chains. Interestingly, these hydrophilic groups of polar side chains could also possess zinc-chelating ability (Sun et al., 2016; Wang,

* Corresponding author at: No. 1 Qinggongyuan, Ganjingzi District, National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, PR China.
E-mail address: linsongyi730@163.com (S. Lin).

http://dx.doi.org/10.1016/j.foodchem.2017.09.025
Received 19 May 2017; Received in revised form 24 July 2017; Accepted 4 September 2017
Available online 07 September 2017

Li, Yang, Wang, & Shen, 2009). Zinc-chelating peptides have been identified from sesame protein hydrolysates, rapeseed protein hydrolysates and oyster protein hydrolysates (Chen et al., 2013; Wang, Li, & Li, 2014; Xie et al., 2015). In addition, the zinc binding sites of peptides were also investigated. The interaction of zinc with peptides occurs mainly through the carboxylate groups of the side chains of Asp and Glu residues, with some contribution of the NH groups belonging to the peptide backbone (Gerbino, Mobili, Tymczyszyn, Fausto, & Gómez-Zavaglia, 2011). The side chain hydroxyl group of Ser, sulfhydryl group of Cys might play important roles in the complexation between peptides and zinc (Wang, Li, & Ao, 2012). These above coordination groups are hydrophilic and may contribute to the hygroscopicity of peptides (Singh, Rao, Anjaneyulu, & Patil, 2001).

In our previous work, an antioxidant pentapeptide, Ser-His-Cys-Met-Asn (SHCMN), was separated and identified by reversed phase (RP)-HPLC and mass spectrometry (LC–MS/MS) from soybean protein hydrolysates treated by alkaline protease (Lin, Liang, Li, Xing, & Yuan, 2016a). In particular, it contains specific amino acids (Ser, His and Cys) associated with zinc binding. This present work investigated whether zinc could bind to the moisture absorption sites of SAP and the effect of zinc chelate on hygroscopicity was also studied in order to elucidate a method of targeted regulation of peptide powder hygroscopicity. This study could provide a reference for the study of methods to regulate the hygroscopicity of peptide powder.

2. Materials and methods

2.1. Materials and chemicals

The SAP, Ser-His-Cys-Met-Asn, derived from soybean protein was synthesized by China Peptides Co., Ltd. (Shanghai, China) by solid-phase peptide synthesis method with 98.85% purity. All of chemicals and reagents used in this study were of analytical grade and commercially available.

2.2. Preparation of SAP-zinc complex

The SAP-zinc complex was prepared according to the method described by Xie et al. (2015) with minor modification. The peptide-zinc complex was obtained after the precipitates were washed with ethanol three times and finally recovered by centrifugation. Afterward, the complex was collected and lyophilized for further analysis.

2.3. Ultraviolet absorption spectroscopy

In order to investigate the main binding sites of zinc on peptides and monitor the occurrence of the binding reaction, ultraviolet (UV) spectra were measured according to the method described by Chen et al. (2013) with some modifications. Samples of SAP or mixture of SAP and $ZnCl_2$ were dissolved in 0.1 M Tris-HCl (pH 6.0) and filtered through 0.22 μm Millipore filters before placing the samples in quartz cuvettes. The absorption spectra were recorded in the 200–400 nm region with a Perkin Elmer spectrophotometer (Lambda 35) using Tris-HCl as a reference.

2.4. Energy-dispersive x-ray spectroscope (EDS) analysis

The elemental compositions of the SAP and SAP-zinc complexes were carried out with an energy-dispersive X-ray spectroscope (EDS, X-MaxN 50) operated at an acceleration voltage of 5 kV. The procedure was performed according to the method described by Voogt, Hirte, and Meinders (2011) with minor modification. In brief, the SAP or SAP-zinc complex powders were placed on an adhesive tape attached to a circular aluminum specimen stub and coated with gold–palladium under 15 mA current.

2.5. X-ray diffraction (XRD) analysis

Wide angle X-ray scattering pattern of SAP or its zinc complex was performed by using an X-ray diffracmeter (XRD-7000S, Shimadzu Corp., Japan) with Cu radiation ($\lambda = 1.54$ Å) at 40 kV and 30 mA. Samples were recorded with the scanning angle (2θ) from 10° to 70° at a scanning rate of 5°/min. The gallery height (d-spacing distance) was determined by the peak in the XRD pattern and expressed by Bragg's equation ($\lambda = 2\,d{\cdot}\sin\theta$).

2.6. Scanning electron microscopy (SEM) analysis

The morphology of SAP and SAP-zinc complexes was studied using a field-emission scanning electron microscope (JEOL JSM-7800F) according to the method as previously described (Sun et al., 2017).

2.7. Fourier transform infrared (FTIR) spectroscopy

The SAP or SAP-zinc powder was mixed with dry KBr. Once ground, the mixture was compressed into a thin disc. The infrared spectra in the spectral range between 4000 and 400 cm^{-1} were recorded with a Perkin Elmer FTIR instrument at a resolution of 4 cm^{-1}. All measurements were performed in a dry atmosphere at room temperature (25 ± 1 °C). The peak signals in the spectra were analyzed using OMNIC 8.2 software (Thermo Nicolet Co., Madison, WI, USA).

2.8. Dynamic vapour sorption (DVS) measurements

Water sorption kinetics of SAP and SAP-zinc complex powders were measured according to methods described previously (Young, Edge, Staniforth, Steele, & Price, 2005) with modification. A humidity controlled microbalance system (DVS apparatus, Surface Measurement System Ltd, London, U.K.) was used to expose powder samples to different RH conditions with a preset parameter ranging from 0 to 90% at a 10% RH change gradient, 90–95% with a span of 5% RH and then in reverse order to 0 RH. Experimental temperature was maintained at 25 °C. Powder samples were laid in the aluminum holder of the DVS chamber. The demanded RH was automatically controlled by a constant dry nitrogen gas flow and another nitrogen gas stream containing water vapour. It was assumed that the equilibrium was reached when the change of dm/dt was lower than 0.002% during consecutive 5 min, or the maintenance time was over 180 min. Then the target RH was automatically jumped to the next preset value.

2.9. Low-field nuclear magnetic resonance (LF-NMR) measurements

Based on the DVS measurements, the SAP and SAP-zinc treated with 93.6% RH (generated by KNO_3 saturated salt solutions) were chosen to conduct the further investigations. LF-NMR measurements were performed by using a NMI20-015V-1 analyzer (Niumag Co., Ltd., Shanghai, China). SAP or SAP-zinc complex powders were put into injection bottles (1.5 mL, Agilent). Then the injection bottles were placed in a sealed chamber at 25 °C under 93.6% RH generated by saturated salt solutions of KNO_3 to facilitate water absorption. Transverse relaxation times (T_2) were measured using the MSE-CPMG sequence and the following parameters: 90° pulse = 7.2 μs, 180° pulse = 14.2 μs, analog gain RG1 = 20 db, digital gain DRG1 = 3 db, preamplifier gain PRG = 3, RFD (the parameter to control the first data point that is acquired) = 0.1 ms, the number of scans NS = 256, the number of echoes NECH = 1, DL10 = 25 ms, SW (the receiver bandwidth frequency) = 200 kHz and TW (the duration between successive scans) = 100 ms. Normalization processing was carried out for T_2 relaxation peak area and amplitude. LF-NMR data analysis and distributed exponential curve fitting were performed according to Yang et al. (2016).