



Cod peptides inhibit browning in fresh-cut potato slices: A potential anti-browning agent of random peptides for regulating food properties

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ABSTRACT

Enzymatic browning is a major industrial problem of fresh-cut vegetables and fruits. Bioactive peptides are safe, nutritive and low-cost sources of antioxidant and antimicrobial agents. However, there has been little research on the effect of random peptides on anti-browning of fresh-cut food. For developing more natural and nutritive anti-browning agents, the effect of the enzymatic hydrolysis of random peptides from cod fish skin on polyphenol oxidase (PPO), peroxidase (POD), phenylalanine ammonia-lyase (PAL), total phenolic content, membrane permeability, malondialdehyde (MDA) content and color changes were investigated during fresh-cut potato storage. The results showed that 0.1% (w/w) cod peptides efficiently blocked enzymatic browning by inhibiting PPO, POD and PAL activities, reducing the total phenol accumulation during the entire 8 d storage at 4 °C. Furthermore, the membrane permeability and MDA content increases were delayed in 0.1% cod peptides treatment compared with the control. Oddly, the browning of fresh-cut potato was aggravated in 1.0% cod peptides treatment, which expressed higher POD and PAL activities. Meanwhile, the functional composition of cod peptides was a random component, which total 1765 peptides were identified by LC-MS/MS and the amino acids length of peptides were 4–57 in the cod peptides. All the results showed that random peptides might be promising candidates as anti-browning agents for fresh-cut potato slices.

1. Introduction

Potato (*Solanum tuberosum* L.) is the third largest food crop worldwide and is a good source of vitamins, minerals and dietary fiber as well as phytochemicals (Ma et al., 2010a, 2010b; Weaver and Marrs, 2013). With the new fast-paced lifestyle, the demands of fresh and ready-to-eat food has increased quickly (over 30% over the past decade) due to its healthfulness, convenience and freshness (You et al., 2012; Harich et al., 2018). Usually, the appearance, texture, flavor, nutrition and safety of the food is related to the quality of fresh-cut produce. Appearance is the main factor affecting consumer choice in the first phase of purchase (Francis et al., 2012). However, fresh-cut products are always susceptible to the process of surface browning, which leads not only to the color changing but also to a shorter of shelf-life and a consumer's purchase decision (Oms-Oliu et al., 2010).

Browning discoloration is one of the main factors limiting the quality of fresh-cut potato slices during storage (Cantos et al., 2002; Ma et al., 2010a, 2010b; Wang et al., 2015). It is widely believed that

enzymatic browning is mainly due to the oxidation reaction of endogenous phenols to quinones by polyphenol oxidase (PPO) or peroxidase (POD) in the presence of oxygen, which is then subjected to further reactions, leading to pigment formation (Cantos et al., 2002; Zhou et al., 2003; Oms-Oliu et al., 2010). Meanwhile, phenylalanine ammonia-lyase (PAL) is another key enzyme for enzymatic browning by substrate generation of phenolic substances, lignins, flavonoids and other secondary metabolites in the phenylalanine pathway (Cantos et al., 2002).

Various anti-browning agent have been used to prevent browning of fresh-cut vegetables and fruit, such as citric acid, ascorbic acid, isoascorbic acid, sodium erythorbate, and various plant essential oil, plant polyphenol extracts, chitosan, and so on can effectively improve the browning degree (Buta et al., 1999; Solivafortuny et al., 2002; Dong et al., 2010; Sapers and Miller, 2010). Among them, several browning inhibitors have been reported that can inhibit PPO, POD, and PAL activities. For instance, citric acid blocks PPO activity by reducing the pH; chelating the copper to the enzyme active site of sulfhydryl-containing

Abbreviations: PPO, polyphenol oxidase; POD, peroxidase; PAL, phenylalanine ammonia lyase; MDA, malondialdehyde

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amino acids such as L-cysteine, which react with quinone intermediates to form stable, colorless compounds (McEvily et al., 1992; Artes et al., 1998; Jiang et al., 1999; Rocculi et al., 2007).

In recent years, it is noteworthy that natural agents that are health-promoting and anti-browning are a new trend. Numerous researchers have demonstrated that hydrolytic peptides and amino acids have beneficial effects on human health through their antioxidant, antibiotic and anti-aging activities, such as control of obesity, high blood pressure and cardiovascular disease (Kadam and Prabhasankar, 2010; Halim et al., 2016). In addition, there has been great interest in bioactive peptides functional food for promoting consumer health and improving the shelf life of food products (Adapa et al., 2000; Najafian and Babji, 2012; Venkatesan et al., 2017; Di Francesco et al., 2018; Gallego et al., 2018; Moayedi et al., 2018; Mune et al., 2018; Yu et al., 2018). Even more important, peptides can be used to interfere with protein functions and protein–protein functions in plant cells as peptide aptamers for a given target (de Chasseay et al., 2007; Hao et al., 2018). Hence, developing the bioactive peptides was relatively safe and promising.

Peptides are potential anti-browning agents because of their good antioxidant function. It was reported that some amino acids or dipeptides significantly inhibit browning of fresh-cut vegetables and fruit (Girelli et al., 2004; Ali et al., 2016a,b; Wills and Li, 2016). However, there is very little research into the effect of random peptides on anti-browning of fruits or vegetables. Meanwhile, although cod peptides, a major high-quality peptide source, exhibit biological activities, there is limited research on the function of cod peptides as a preservative. Here, we investigated the anti-browning characteristics of cod peptides in fresh-cut potato slices through the evaluation of PPO, POD, PAL, total phenol content, MDA and other related indicators.

2. Materials and methods

2.1. Material and sample preparation

Potato tubers (*S. tuberosum*, cv Netherlands 7) were obtained from a local Agricultural Market in Tianjin, China. These potato tubers were fresh, uniform in size and those free from mechanical damage were selected for processing into fresh-cut slices. The selected potatoes were washed, peeled and cut into thin slices with a thickness of 0.5 cm (Ma et al., 2010a,b). And the cod peptides were obtained from Qingdao Ruikang Biotechnology Co. Ltd.

Then, the fresh-cut slices were divided randomly into four groups, which were immersed in distilled water (control) or a solution of cod peptides with concentrations of 1%, 0.1% and 0.01% (w/w) for 5 min at room temperature with a ratio of grams of potato tissue to milliliters of solution 1:4. Subsequently, all treated fresh slices were carefully drained by gauze then packed into a polyethylene (PE) zip lock bag. Finally, they were stored at 4 ± 1 °C for 8 d in a mini cold storage room, and used for the following experimental measurements. For each treatment, the experimental measurements were taken every two days and every time three replicates whole bags from each treatment were randomly selected to investigate and analysis in the next experiment.

2.2. Color analysis

The surface color of the potato slices was determined using a colorimeter (HP-200, Shanghai Chinaspec optoelectronics Technology CO., LTD, Guangdong, China). The L (lightness), a (reddish–greenish) and b (yellowish–bluish) indexes of the CIELAB colorimetric system were used to evaluate the color change of the potato samples (Zhou et al., 2015). The slices and each side of every slice were measured for every treatment. Total color differences (ΔE^*) were compared to time zero, and calculated according to the following formula: $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$.

2.3. Enzymes activity analysis

2.3.1. PPO activity assay

PPO activity was measured according to a previously described method with slight modifications (Galeazzi et al., 1981). First, 5.0 g of fresh potato slices were homogenized in 5.0 mL of 0.1 mol L⁻¹ sodium acetic acid sodium acetate buffer (pH 5.5). After centrifugation at 16,000 × g for 30 min, the supernatant was collected for PPO activity measuring. The supernatant (100 μL) was reacted with 4.0 mL of 0.5 mol L⁻¹ acetic acid-sodium acetate buffer (pH 5.5) and 1.0 mL of 0.5 mol L⁻¹ catechol solution. Immediately, the absorbance at 420 nm was measured per minute. The activity of PPO was calculated using the formula of reference and expressed in U g⁻¹ fresh weight.

2.3.2. POD activity assay

POD activity was measured by a previous method with slight modifications (Kochba et al., 1977). A total of 5.0 g fresh potato slices were ground into a homogenate with 5.0 ml buffer (0.1 mol L⁻¹ sodium acetic acid sodium acetate buffer, pH 5.5). After centrifugation at 16,000xg for 30 min, the supernatant was collected as the potato enzyme extract. The mixture, which consists 0.5 mL of the enzyme, 3 mL of 50 mmol L⁻¹ boric acid buffer (pH 8.8) and 0.5 mL of 20 mmol L⁻¹ l-phenylalanine solution was incubated at 37 °C for 10 min. After 200 μL of 0.5 mol L⁻¹ H₂O₂ was added into the mixture, the absorbance at 470 nm was measured every 1 min for a total of 6 min. POD activity was expressed as U g⁻¹ fresh weight.

2.3.3. PAL activity assay

PAL activity was measured according to a modified previous method (Assis et al., 2001). The 0.5 mL of enzyme extract was blended and incubated at 37 °C for 10 min with 3 mL boric acid buffer (50 mmol L⁻¹, pH 8.8) and 0.5 mL l-phenylalanine solution (20 mmol L⁻¹). Then, the absorbance of 290 nm was measured at 0 min and 60 min. The activity of PAL was expressed on a fresh weight basis as U g⁻¹ fresh weight.

2.4. Membrane permeability

Membrane permeability assays were conducted as described previously with minor modifications (Zhang et al., 2005), which is expressed as the relative leakage rate. Firstly, ten potato slice discs of 10 mm diameter were washed three times by deionized water. After drying with filter paper, these potato slices were put into a test tube with 20 mL deionized water for 30 min. Then the test tube was boiling 15 min and quickly cooling to room temperature. Finally, the electrical conductivity was measured by a conductivity meter (Model 3173, Shanghai Electronics Co., Ltd., China).

2.5. Malondialdehyde (MDA) content analysis

MDA content was measured according to the previous method (Dhindsa et al., 1981) with slight modifications. Fresh potato slices (5.0 g) were homogenized with 10 mL trichloroacetic acid (TCA, 100 g L⁻¹) and a small amount of quartz sand. After centrifugation at 16,000 × g for 10 min, the supernatant was mixed with 3 mL TCA (0.6%, w/w), and incubated in boiling water for 15 min. And then, cooling and then centrifugation at 1000 xg for 15 min, the supernatant was mixed with 0.06% (w/w) thiobarbituric acid (TBA) solution. Finally, the absorbance of the mixture was measured at 532 nm, 600 nm, and 450 nm. MDA content was expressed on a fresh weight basis as mmol kg⁻¹.

2.6. Total phenolic content analysis

Total phenolic content was determined by the Folin-Ciocalteu procedure according to the previous report (Piccolella et al., 2008) with

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