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Applicative effect of glycinin basic polypeptide in fresh wet noodles and antifungal characteristics

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ABSTRACT

We aimed to evaluate applicative effect of glycinin basic polypeptide (GBP) on fresh wet noodles during 5 days storage at 4 °C and to investigate its antifungal characteristics against *Aspergillus niger* (*A. niger*) and *Penicillium* sp.. GBP enhanced the sensory scores for fresh wet noodles and reduced the total plate count of fungi in a dose-dependent manner during storage. More than 2.0 mg/g GBP had well preservative effect on fresh wet noodles with 4 days shelf life at 4 °C. The inhibition zone diameters for *A. niger* and *Penicillium* sp. increased with increasing GBP concentration. The minimum inhibition concentrations of GBP against *A. niger* and *Penicillium* sp. were 2.0 and 1.5 mg/mL, respectively. GBP could effectively inhibit mycelial growth and spore germination of *A. niger* and *Penicillium* sp.. The ergosterol content (EC) in the fungal plasma membrane decreased with the increase in GBP concentration. What is more, EC of fungi with 2.4 mg/mL GBP had significant different from other concentrations of GBP ($p < 0.05$). Thus, GBP had remarkable antifungal effects by inhibiting growth of mycelium and spore germination, and disrupting the plasma membrane of fungi. GBP might be an alternative to synthetic antifungal preservatives in starchy food to extend shelf life.

1. Introduction

Fresh wet noodles are becoming increasingly popular worldwide for their convenience, nutritional quality, and palatability. However, fresh wet noodles have a short shelf-life because they are easily contaminated by fungi, especially *Aspergillus niger* (*A. niger*) and *Penicillium* sp. due to the high contents of water and starch (Li et al., 2012). Various synthetic antifungal preservatives are applied to fresh wet noodles such as benzoyl peroxide and potium sorbate. Exposing benzoyl peroxide to wheat flour whitened it and brightened its color and served as an antifungal agent to extend its shelf life (Her, Jones, & Wollack, 2014). However, the application of synthetic preservatives in food might harm human health because of carcinogenicity, teratogenicity, and toxicity concerns (Meyer, Suhr, Nielsen, & Holm, 2002). For example, irritant odor of benzoyl peroxide could harm human respiratory tract. Moreover, overuse of benzoyl peroxide oxidized vitamin E and B, and reduced the nutritional value of food (Her et al., 2014). Now it is prohibited to use in China (GB2740-2014, China). Therefore, safe natural food preservatives have become a priority for human consumption and food industry (Najjar, Kashtanov, & Chikindas, 2007).

Natural antimicrobial peptides, originating from bacteria, plants, and animals, play an important role in food preservatives because of safety to human health (Tang et al., 2015). Some natural antimicrobial peptides exhibited antimicrobial activities against fungi (Falcao et al., 2016). Lima bean peptide had antifungal effect on various fungi by measuring the diameters of the inhibition zone (Wang & Ng, 2006). A new crude extract peptide of *Cassia tora* showed antifungal activity against *A. niger*, with minimum inhibition concentration (MIC) of 2.5 mg/mL (Gahlaut, Dabur, & Chhillar, 2013). At 1–10 μg/mL, recombinant antifungal defensin could inhibit the mycelial growth of wild-type *Candida albicans* cells in *Brassica juncea* (Oguro, Yamazaki, Takagi, & Takaku, 2014).

GBP is a basic subunit of soybean glycinin stemming from natural soybean. It is linked to the acid subunit of soybean glycinin by a

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single disulfide bond. GBP is a cationic peptide of approximate 20 kDa (Shin, Park, Park, & Kim, 2007). Several investigations reported GBP had antimicrobial activities against some gram-positive and gram-negative bacteria (Sitohy, Mahgoub, & Osman, 2012). GBP could suppress the growth of *Listeria monocytogenes*, *Salmonella* enteritidis and *Bacillus subtilis* inoculated in pasteurized milk after 16–20 days at 4 °C. The antimicrobial mechanism might be the interaction of GBP with the bacterial cell wall and membrane on account of its cationic and hydrophobic nature (Sitohy et al., 2012). Osman, Mahgoub, and Sitohy (2013) reported that the soybean 11S subunit could retain good-quality characteristics of bovine raw milk and extend the storage time at room temperature and under cold conditions. In previous reports, GBP damaged the structure of the phospholipid bilayer of bacterial membranes, enhanced the cell membrane permeability, and released proteins and ions from *Escherichia coli* cells, then hampered cell growth and propagation and even resulted in cell death (Li, Sun, Feng, & Mo, 2015). The application of GBP to pasteurized milk and chilled pork could improve sensory properties and reduce the total plate count (TPC) of bacteria, thereby extending the shelf life (Li, Hao, Yang, & Mo, 2016; Zhao, Li, Sun, & Mo, 2016).

To date, there are no investigations about antifungal characteristics of GBP, especially *A. niger* and *Penicillium* sp.. The two fungi are usually considered indicator fungi in starchy food because of their easy contamination to these foods. Here we studied antifungal characteristics of GBP against two fungi and the application of GBP to fresh wet noodles, which are aboundant in starch. The applicative effect of GBP on fresh wet noodles was estimated by sensory scores and TPC of fungi. The antifungal activity was examined by MIC and the Oxford cup test. The antifungal action of GBP against *A. niger* and *Penicillium* sp. was elucidated by changes in mycelial growth, spore germination, and ergosterol content in the plasma membrane of the two fungi.

2. Materials and methods

2.1. Preparation and purification of glycinin basic polypeptide (GBP)

Defatted soybean flakes, purchased from Scents Holding Co. Ltd. (Jinan, Shandong), were ground and passed through a sieve (1 mm^2). The obtained powder was used for isolating glycinin according to the method reported by Nagano, Hirotsuka, Mori, Kohyama, and Nishinarit (1992). The isolated glycinin was dissolved in 30 mM Tris buffer (pH 8.0, containing 15 mM β-mercaptoethanol) to a final concentration of 2% (w/v). The glycinin solution was heated to 90 °C for 30 min and cooled to 4 °C, then centrifuged (11,000*g*) for 15 min at 4 °C. The precipitate (crude GBP) was washed three times with 30 mM Tris buffer (pH 8.0), dispersed into distilled water and freeze-dried. The crude GBP was dissolved in 10 mM phosphate buffer (pH 7.2) to a final concentration of 1 mg/mL. Then 1 mg/mL GBP was added to Sephadex G-150 (Troody Co. Ltd., Shanghai, China) and eluted with the same phosphate buffer (pH 7.2) at 2 mL/min. The eluent containing purified GBP was collected and freeze-dried to powder for the experiments.

2.2. Preparation of fresh wet noodles with GBP

The fresh wet noodles were prepared using the method described by Li et al. (2012). GBP powder was dispersed in 60 mL water and the dispersion of GBP was added to 200 g wheat flour to obtain final concentrations (w/w) of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/g. The dough was formed by using a Kitchen Aid mixer (BO-C02, Beow Instrument Co. Ltd., Guangzhou, China) and rested in a plastic bag for 20 min. The prepared dough was passed through a small noodle machine (DHH180, Haiou Instrument Co. Ltd., Zhejiang, China) several times with the roller gap to obtain dough sheets. Resulting dough sheets were cut into fresh wet noodles with 2.0 mm thick and 5.0 mm wide. Fresh wet noodles were stored at 4 °C.

2.3. Sensory evaluation of fresh wet noodles

The sensory properties of fresh wet noodles were evaluated as described (Küçükgülmez, Kadak, & Gökçin, 2013; Ma, Fu, Xu, Trebar, & Zhang, 2016) by seven panelists trained to be familiar with the characteristics of noodles. Fresh wet noodles treated with 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/g GBP were cooked for about 4 min. The panelists scored the sensory properties of fresh wet noodles, including appearance, odor, taste, texture, and overall acceptability, on a 9-point descriptive scale (1, dislike extremely, 2, dislike very much, 3, dislike, 4, slightly dislike, 5, neither like nor dislike, 6, like a bit, 7, like, 8, like very much, 9, like extremely).

2.4. Measurement of total plate count (TPC) of fungi

TPC of fresh wet noodles stored at 4 °C was determined as described (Li et al., 2012) with some modification. About 25 g fresh wet noodles were added to 225 mL sterile saline and mixed by using a homogenizer (Scientz-09, Scientz Co. Ltd., Shanghai, China). Then the mixture was placed in a 500 mL flask to obtain 10% sample dispersion. The potato-glucose agar medium (300 g potatoes, 20 g glucose, and 20 g agar dissolved in 1000 mL distilled water) was supplemented with chloramphenicol and chlortetracycline HCL antibiotics finally to obtain 5 g/L in potato-glucose agar medium (Association of Official Analytical Chemists 2000). Serial dilutions of the sample dispersion (1.0 mL) in sterile distilled water were mixed with the potato-glucose agar medium in Petri plates and incubated at 28 °C for 3 days in an incubator (SHP-150, Jinghong Experiment Equipment Co. Ltd., Jinan, China). The TPC was calculated by the colony-counting method.

2.5. Preparation of Aspergillus niger (A. niger) and Penicillium sp. spore suspension

These two strains were isolated from fresh wet noodles and purified according to the following methods of Mahilrajan, Nandakumar, Kailayalingam, Manoharan, and SriVijeindran (2014) and Mehyar et al. (2011). Selected two fungi were identified as *A. niger* and *Penicillium* sp. in based on macroscopic and microscopic features, which conformed to those of *A. niger* and *Penicillium*. Then, *A. niger* and *Penicillium* sp. were incubated at 28 °C for 3 days in solid Czapek Dox medium (1 g sodium nitrate tryptone, 0.5 g dipotassium phosphate, 0.025 g potassium chloride, 0.005 g magnesium sulfate, 0.005 g ferrous sulfate, 15 g sucrose, and 9 g agar dissolved in 1000 mL distilled water) to obtain fungal spores. The plate medium containing spores was mixed with 10 mL sterile distilled water to obtain spore suspension, which was filtered through five layers of sterile degreased cotton to remove hyphae. Cells in the filtered spore suspension were counted by using a hemocytometer (201312, Qiujing Co. Ltd., Jinan, China) (Bajpai, Shukla, & Kang, 2008).

2.6. Measurement of inhibition zone diameter

GBP powder was dispersed in sterile water to a final concentration (w/v) of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/mL. Antifungal activities of GBP against *A. niger* and *Penicillium* sp. were determined by the Oxford cup method (Wang, Lu, Wu, & Lv, 2009) with some modification. Aliquots of 100 μL (approximately 10^6 spores/mL) of the spore suspension were spread uniformly onto the