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Optimization of liquid fermentation conditions for biotransformation zein by *Cordyceps militaris* 202 and characterization of physicochemical and functional properties of fermentative hydrolysates

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ABSTRACT

Cordyceps militaris 202 is a potential fungus for biotransformation zein, due to its various proteases, high tolerance and viability in nature. In this article, single factor experiment and response surface methodology were applied to optimize the liquid fermentation conditions and improve the ability of biotransformation zein. The optimized fermentation conditions were as follows: inoculum concentration of 19%, volume of liquor of 130 mL/500 mL and pH of 4.7. Under this condition, the degree of hydrolysis (DH) was 27.31%. The zein hydrolysates from fungi fermentation maintained a high thermal stability. Compared to the original zein, the zein hydrolysates were found to have high solubility, which most likely results in improved foaming and emulsifying properties. Overall, this research demonstrates that hydrolysis of zein by *C. militaris 202* is a potential method for improving the functional properties of zein, and the zein hydrolysates can be used as functional ingredients with an increased antioxidant effect in both food and non-food applications.

Introduction

Zein is the major proportion (65%) of corn gluten meal (CGM), and CGM is major co-product of corn wet-milling process. China is the second largest country for production and consumption of corn. Zein is excellent materials with unique nature, and its poor solubility in water, which contributes to zein difficult in food industry application.[1] Protein modification may be carried out to improve its nutritional and/or functional properties. Modification of protein usually contain physical,[2] chemical[3] and biological treatments,[4,5] which change the physicochemical and functional properties by changing its conformation and structure. This has

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been reviewed by several researchers.[6–8] The biological treatment is one of the most used methods among the researches for modification, which consists of enzyme treatment and fermentation. The hydrolysates may be expected to have increased solubility, foaming and emulsifying properties compared with native protein, and there are many studies have proved these.[9–11] Compared with the single enzyme hydrolysis, the hydrolysis by liquid fermentation has advantage on several aspects, such as low cost, and food safety, thus making it more suitable for protein modification. Zhang[12] thought that the product of polypeptide by liquid fermentation can save 40–50% cost, compared with enzyme hydrolysis.

Cordyceps militaris is considered as one of the most expensive mushrooms in China, because of its attractive beneficial effects and rarity in nature.[13] The submerged culture for producing mycelium can be used as ingredients in functional foods, which have conduct to meet the demand for commercial applications.[14–16] Xiao[17] found the *C. militaris* can degrade chickpea protein and improve water holding capacity, fat absorption capacity and emulsifying properties of flours by solid-state fermentation. The utilization of liquid fermentation of zein with *C. militaris 202* can transform zein into soluble protein, thus improving the utilization and bioavailability in the aqueous phase. However, the existent researches about *C. militaris* mostly focus on its metabolite,[18–20] no available reports about use of enzymatic proteolysis by *C. militaris 202* for modifying the functionality of zein could be found.

Thus, our subject is to optimize the fermentation condition of transforming zein to soluble protein by liquid fermentation with *C. militaris 202*. The degree of hydrolysis (DH) was used as the evaluation to obtain optimal fermentation conditions by response surface methodology (RSM). Moreover, we investigated the effects of fermentation time on the physicochemical and functional properties (solubility, emulsification, foaming properties and antioxidative activity) of zein hydrolysates. The information is essential in modifying zein by liquid fermentation, which can provide a potential method to improve function properties and biological activity of zein, and improve its utilization value and field.

Materials and methods

Materials

Zein was obtained from Shanghai Ryon Biological Technology, China (purity > 92%). *C. militaris 202* was preserved in National Engineering Laboratory for Wheat and Corn Deep Processing. All other chemicals were of analytical grade

Media

Three media were used in this experiment, as follows: PDA, fermentation seeding, fermentation media. The composition of three media was as follows:

PDA medium (g/L): potatoes 200.0, glucose 20.0 and agar 18.0.

Fermentation seeding (g/L): potatoes 200.0, glucose 20.0, $MgSO_4$ 1.5, KH_2PO_4 3.0, V_{B1} 0.2.

Fermentation media (g/100 mL): glucose 7.0, zein 6.0, $MgSO_4$ 0.15, KH_2PO_4 0.3, V_{B1} 0.2.

Methods

Preparation of liquid fermentation

C. militaris 202 was incubated in the agar medium at 24 °C for about 7 d, until the white mycelia covered on the surface of the agar culture media. Three truffles were inoculated in 100 mL conical flasks containing 30 mL liquid medium. Mediums had been sterilized at 121 °C for 20 min before inoculation (YXQ-LS-SII, Boxun Corp, Shanghai, China). The conical flasks were kept at 24 ± 1 °C for 3 d, and then were used as the *C. militaris 202* seeding culture for zein fermentation. *C. militaris 202* seeding was broken using a sterilized Blender, then inoculated 10 mL seeding [equivalent to 10% of the fermentation medium (100 mL)] to 500 mL conical flasks, the conical flasks were shaking with 160 r/min at 24 °C for 8 d.

Optimization of zein hydrolysates fermentation conditions

The fermentation conditions were optimized to improve degree of hydrolysis (DH), including inoculum concentration (v/v, 5%, 10%, 15%, 20%, 25%), volume of liquor (equivalent to total volume of the fermentation medium) (mL/mL, 80/500, 100/500, 120/500, 140/500, and 160/500), pH (2.0, 4.0, 6.0, 8.0, 10.0). The biomass and degree of hydrolysis were determined. The experiments were conducted in three replicates.

Biomass

The mycelia in the fermented broth were washed for 4 times by distilled water, and then were obtained by filtering using absorbent gauze. The mycelia was dried to a constant weight in the hot air oven at 105 °C (YLA-6000, Shanghai, China), then measuring dry weight for biomass. The experiments were conducted in three replicates.

Degree of hydrolysis (DH)

The DH was measured according to the method of Yang.[21] The supernatant of fermented broth was obtained by centrifugation, then, mixing 15 mL 10% trichloroacetic acid solution (TCA) with 15 mL supernatant, and then the mixture was put at 25 °C for 30 min. The sediment was removed by centrifugation at 4000 × *g*. The soluble nitrogen in supernatant and total nitrogen content of substrate were determined by microKjeldahl. The experiments were conducted in three replicates. The DH was calculated as follows:

$$DH = \frac{X - X_0}{S_0 - X_0} \times 100\% \quad (1)$$

DH: degree of hydrolysis

X: soluble nitrogen content in 10% TCA of fermented liquor, g

X_0: soluble nitrogen content in 10% TCA of unfermented liquor, g

S_0: total nitrogen content of substrate, g

Preparation of zein hydrolysates by liquid fermentation

After fermentation under the optimized fermentation conditions (optimized by RSM), the reaction was terminated by boiling for 5 min. In order to extract the zein hydrolysates, the fermented broth was centrifuged at 4000 × *g* for 20 min and the zein hydrolysates were obtained by fraction precipitation

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