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Original Article

Separation and speciation analysis of zinc from *Flammulina velutipes*

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ABSTRACT

Orthogonal experiment was applied to optimize the water extraction parameters of zinc from *Flammulina velutipes*, and then the extracts were separated by membrane filter (0.45 μm) and D101 macroporous resin. Six different species of Zn were obtained and the Zn content of various species were determined by flame atomic absorption spectrometry. The optimized conditions for the extraction of Zn were: ratio of dried material to water, 1:30; extraction temperature, 75°C; extraction time, 120 minutes. About 34.43 μg Zn was extracted from 1 g dried *F. velutipes* powder under the optimal conditions. The recovery value for Zn was 96.5% with a low relative standard deviation. In addition, the content of the organic state of Zn was more than that of the inorganic state, and most of the organic state Zn was found in the polysaccharide and protein fractions.

1. Introduction

Flammulina velutipes is becoming a popular food for its immune-modulating, anti-oxidation and antitumor functional effects [1–4]. Along with the development of research, *F. velutipes* is well known for nutritional ingredients (e.g. polysaccharides and proteins) and health benefits [5]. It is also rich in mineral elements, especially the trace elements (e.g. zinc, chromium, and copper), which have strong physiological functions, and numerous medicinal and therapeutic effects of trace elements have been reported [6–8]. For example, Zn is essential for neurogenesis, synaptogenesis and neuronal growth [9], Cu is necessary for enzyme activation, while Cr plays an important role in the function of the pancreatic hormone insulin [10,11]. The physiological function is not only the focus of research, but also a basis of new product development. Accurate determinations of these elements are important for their study [12].

Zn, one of the essential trace elements in the human body, is helpful for health [13]. Zn benefits the nervous system: a deficiency in Zn has been associated with altered neurodevelopment and affects infant behavior, and cognitive and

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movement abilities [14,15]. *F. velutipes* is rich in Zn and approximately 35% of total Zn is combined with polysaccharides [16]. Therefore, it is important to study the chemical speciation of Zn in *F. velutipes* [17].

Some studies have been carried out on the enhancement of Zn concentration, and previous studies focused on the bioaccumulation capacity of elements [18–20]. Rabinovich et al studied the bioaccumulation capacity in mushrooms for the purpose of obtaining organisms enriched in certain essential minerals for human health [21]. Elless et al evaluated the possibility of mushrooms as a natural source of minerals supplements and the potential bioavailability of Zn was also researched [22,23]. However, only a few investigations on different species of Zn in *F. velutipes* have been reported. Specifically, there is a little information about the relationship between Zn speciation and its function. However, speciation information of Zn is very important since the biological role of any particular element greatly depends on its chemical form [24]. It can give useful explanation for the pharmacological mechanism to study Zn content, relative ratio of different species, and dissolution characteristics. Therefore, the objective of this study was to optimize the Zn extraction conditions for the water extraction procedure, including extraction time, temperature and solid–liquid ratio, and to separate and analyze its chemical speciation in *F. velutipes*. D101 macroporous resin was used as a retaining material in the column. The analytical parameters for the quantitative recoveries of analyte ions, such as pH, amounts of reagents, sample volume, were also investigated and six different species of the decoction were obtained. The trace element Zn in the species was determined by microwave digestion-flame atomic absorption spectrometry (FAAS) and the various forms of distribution and chemical speciation were studied [25].

2. Materials and methods

2.1. Material

F. velutipes was purchased from the local market (Nanjing, China). After drying in an oven (DHG-9030A, Shanghai, China) at 50°C for 1 day, *F. velutipes* was ground using a grinder (WFJ-15, Suzhou, China) to pass through a 200 mesh screen. The powder stored at 4°C until used.

2.2. Extraction of Zn

F. velutipes powder (2 g) was solubilized in distilled water at increasing dried material to water ratio (1:10–1:60). The extraction process was performed using a water bath set at increasing time periods (30–130 minutes) and at increasing temperature (within ±1.0°C, 50–100°C). On the basis of single-factor test results (not given in this study), an L_9 (3 × 3) orthogonal test was used in this study, and the variables include extraction temperature, extraction time, and dried material to water ratio. The parameters of L_9 (3 × 3) orthogonal test are shown in Table 1. The experiment was done in triplicate.

The extract was centrifuged (4480×g, 15 minutes), and the supernatant was filtered through a Whatman Nr.1 filter paper, then the filtrate was concentrated using a rotary evaporator at 50°C under reduced pressure to obtain the extracting solution. The Zn extraction ratio (μg/g dried *F. velutipes* powder) was calculated as: Zn extraction ratio (μg/g) = M_0/M, where M_0 (μg) is the Zn content in different forms; M (g) is the weight of dried *F. velutipes* powder.

Table 1 – Orthogonal test results for Zn in *Flammulina velutipes*.

Level	Temperature (°C)	Time (min)	Ratio of dried material to water (w/v)
1	65	100	1:25
2	70	110	1:30
3	75	120	1:35

2.3. Separation of different speciation of Zn

The analytical procedure for different fractions of the sample is presented in Fig. 1.

2.3.1. Preparation of resin

D101 macroporous resins have found widespread application in the separation of organic and inorganic forms of trace metal ions [26]. With the macroreticular structures and high surface area, D101 macroporous has excellent physical, chemical, and thermal stability, and is good for the removal of a variety of metal ions [27]. In this study, D101 macroporous resin from Dingbei Bio-Tech Co. (Nanjing, China) was soaked in 95% aqueous ethanol for 24 hours, and washed thoroughly with deionized water for the next experiment.

2.3.2. Separation of the soluble and the suspended forms of Zn

Dried powder was mixed with water extracting solution for 120 minutes at 75°C and then solution was filtered with 0.45-μm filters, and soluble and suspended species were obtained in the solution and the precipitate separately.

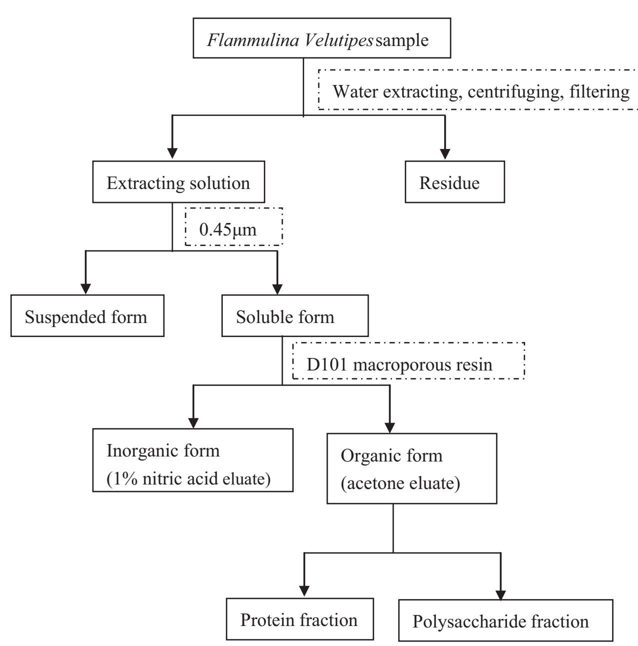

Fig. 1 – Analytical procedure for Zn speciation in samples.