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Short Communication

Extraction of proteins from yeast cell wall

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The proteins from yeast cell wall were extracted by autolysis and depositing in turn. The results show that the change of pH value greatly affects the yield of the final product. The content of obtained crude proteins is maximal (more than 66%) when the autolysis time is 3 h, and pH value is 6 - 7. The extracted proteins can be of social and economic benefits.

Key words: Yeast cell wall, proteins, extraction, autolysis, depositing.

INTRODUCTION

Many evidences have indicated development of new biotechnology (both experimental and computational), such as protein subcellular location prediction (Chou and Shen, 2008), protein quaternary attribute prediction, protein folding rate prediction, identification of membrane proteins and their types, identification of enzymes and their functional classes, identification of G protein-coupled receptor (GPCR) and their types, identification of proteases and their types, protein cleavage site prediction, signal peptide prediction, and protein 3D structure prediction based on sequence alignment (Chou, 2004), which can timely provide very useful information and insights for both basic research and drug design, and hence are widely welcome by science community.

The yeast cell wall is a non-specific stimulator of the immune system of both man and animals (Cabib et al., 2001). It is also applied in the wine industry: its ability to bind undesirable components allows it to prevent and cure stuck fermentations. The yeast cell wall has good thermo-stability, chemical stability, and tolerance against extreme processing conditions. Having no drug fastness, no residual, no pollution, no toxicity and side effect, the yeast cell wall is the pure natural green feed additive with high efficiency for the 21st century. It is a complex structure consisting mainly of proteins, mannan oligo-saccharides, β-glucan, and chitin (Huang et al., 2004; 2005). Most of the proteins are linked to the mannan oligosaccharides and are referred to as the mannoprotein complexes. The proteins have immunoreactivity and biological activity (Cabib et al., 1998). Based on the important biological functions of proteins, the present study is attempted to develop a novel method for extracting proteins from yeast cell wall, and it is hoped that it may become a useful tool for basic research and drug discovery.

MATERIALS AND METHODS

General

Yeast cell walls were purchased from Angel Yeast Co., Ltd (Yichang, China). The concentration of protein was determined by UV absorption using the relationship: $C = 1.45A_{280} - 0.74A_{260}$, where A_{280} and A_{260} are the absorbances of UV at 280 and 260 nm, respectively. This method will correct for any interfering absorbance due to nucleic acid present in the solution (Layne, 1957). IR spectra were recorded with an FT-IR apparatus, and wave numbers are reported in cm^{-1}.

Extraction of proteins

The yeast cell walls were autolyzed with HCl or NaOH solution at 55℃ for 2-4 h, and the pH values ranged from 1 to 12, respectively. The autolyzed mixture was centrifuged at 500 × g for 30 min to remove suspended water-insoluble substances. The pH value of the supernatant liquid was adjusted to 4.3 with NaOH or HCl solution, and the crude proteins were deposited with 80% (v/v) ethanol. The crude proteins were washed 2 - 3 times with 80% (v/v) ethanol again, and freeze-dried to obtain the final product.

RESULTS AND DISCUSSION

The optimization of extraction condition

Proteins from yeast cell wall were extracted by autolysis-

Table 1. The protein contents in crude proteins from different experiments.

Expt. No.	pH value	Autolysis time (h)	A_{280}	A_{260}	Concentration of proteins (mg/mL)	Protein quantity (mg)	Crude protein quantity (mg)	Protein content (%)
1	1 - 2	3	2.370	2.843	1.333	6.665	15.1	44.13
2	6 - 7	3	2.825	2.965	1.903	9.515	14.3	66.52
3	11 - 12	3	2.933	2.933	2.082	10.41	18.9	55.09
4	6 - 7	2	2.738	2.900	1.825	9.125	15.5	58.87
5	6 – 7	4	2.666	2.801	1.793	8.965	14.3	62.69

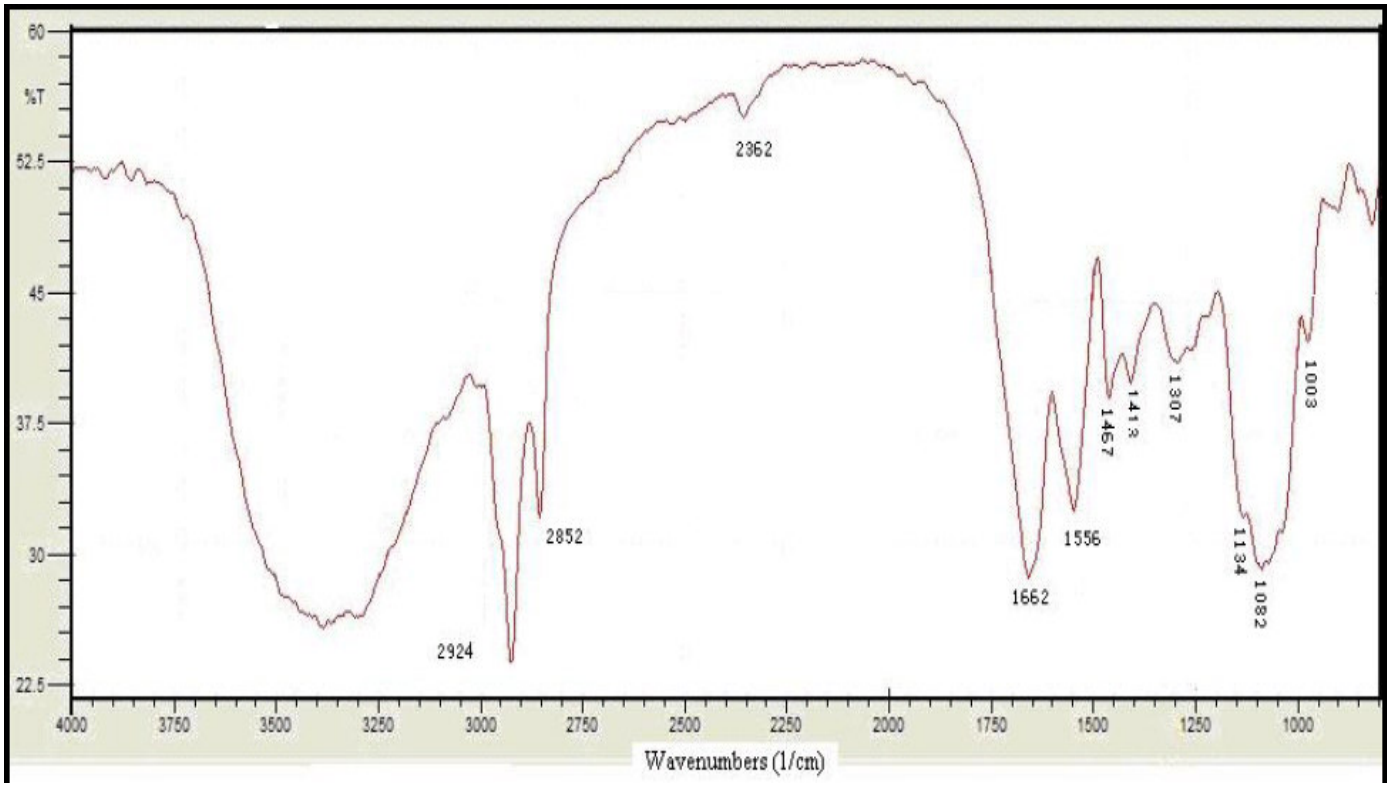

Figure 1. IR spectrum (in KBr) of extracted proteins.

depositing method. The autolysis time and pH value greatly affected the yield of the final product, so the different experiments were designed to study the optimal extraction condition. The experimental results are shown in Table 1. It indicated that the protein content was maximal (up to 66.52%) in Expt. No. 2. This can be explained that the proteins are easy to hydrolyze into peptides with low polymerization degree. So, the optimal condition for extracting proteins is that the autolysis time is 3 h, and pH value is 6 - 7.

The analysis of extracted proteins

The infrared (IR) spectrum of proteins has been observed between 800 and 4000 cm^{-1}. The IR spectrum of extracted proteins (Figure 1) shows the typical spectral pattern of protein, that is to say, it contains the amide bands I (C=O stretch vibration, at 1662 cm^{-1}), II (N-H bend vibration and C-N stretch vibration, at 1556 cm^{-1}), and III (C-N stretch vibration, N-H bend vibration, at 1307 cm^{-1}). The UV absorption spectrum of sample (Figure 2) indicates that the absorbance of UV is at 280 nm, and it proves that the sample is protein.

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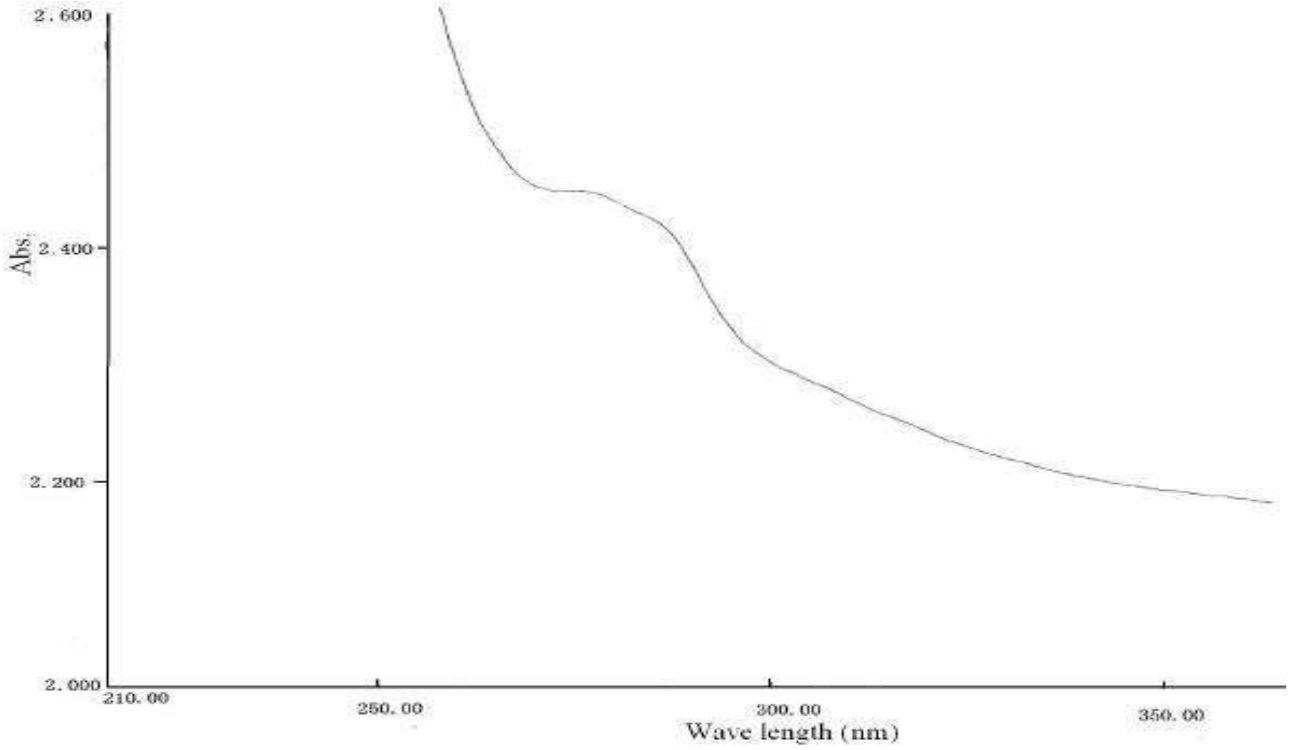

Figure 2. The UV absorption spectrum of extracted proteins.

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