Extraction and Deproteinization of Mannan Oligosaccharides

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Extraction of mannan oligosaccharides from the yeast cell wall and methods of deproteinization were studied. We extracted crude mannan oligosaccharides by the dilute alkali-Sevage method. The percentages of deproteinization and mannan oligosaccharide loss were compared as indexes using the Sevage method, trichloroacetic acid method, and hydrochloric acid method. The results showed that the hydrochloric acid method exhibited the highest percentage of deproteinization, but only a little higher percentage of mannan oligosaccharide loss than the other two methods.

Key words: Mannan Oligosaccharides, Extraction, Deproteinization

Introduction

The yeast cell wall is a non-specific stimulator of the immune system of both man and animals. It is also applied in the wine industry: its ability to bind undesirable components allows to prevent and cure stuck fermentations. The yeast cell wall is made of 30–60% polysaccharides (β-glucan and mannan oligosaccharides) (Huang *et al.*, 2004, 2005), 15–30% proteins, 5–20% lipids, and a small amount of chitin. Most of the protein is linked to mannan oligosaccharides and is referred to as the mannoprotein complex. β-Glucan can stimulate the cells of the immune system (macrophages) and helps to overcome bacterial infections. Mannan oligosaccharide has been demonstrated to prevent diarrhoea in weaning pigs. It binds to pathogenic bacteria in the gut and carries them through and out of the intestinal tract. Mannan oligosaccharide also has prebiotic activity and can serve as a nutrient source for the growth of beneficial bacteria in the colon. Based on the important biological functions of yeast cell walls, the extraction of crude mannan oligosaccharides and methods of deproteinization have been studied. The aim of the present work is to provide an appropriate approach to obtain more pure mannan oligosaccharides. Three methods of deproteinization were investigated, including the Sevage method, trichloroacetic acid (TCA) method, and hydrochloric acid method.

Results and Discussion

IR spectra analysis of enriched mannan oligosaccharides deproteinized by three methods

The mannoprotein from yeast cell wall matrix particles is alkali-soluble (Fujii *et al.*, 1999). So, the crude mannan oligosaccharides were extracted by the dilute alkali-Sevage method. Fig. 1 shows the IR spectra of pure mannan oligosaccharides. They contain absorption bands arising from the ν(CC) and the ν(COC) stretching vibrations at 1152 cm^{-1}, bands at 802 cm^{-1}, 808 cm^{-1}, and 800 cm^{-1} assigned to the corresponding α-mannosidic (C_1–H) deformation mode, and the highest intensity of the ν(OH) bands at lower frequency (3425 cm^{-1} and 3416 cm^{-1}). The presence of carbonyl group bands at 1637 cm^{-1} and 1651 cm^{-1} proved to have the residual protein (10.2%, 8.6%, and 3.3%) in the mannan oligosaccharide samples.

Comparison of the three methods for deproteinization

The Sevage reagent, TCA, and hydrochloric acid as the reagents were investigated for deproteinization of crude mannan oligosaccharides. The principle of the Sevage method is that the dissociative protein is denatured by an organic solvent to an insoluble substance, and removed by centrifugation. The principle of the TCA method is that the protein cation can bind the TCA to form an insoluble salt for precipitation at pH < pI (isoelectric point). The hydrochloric

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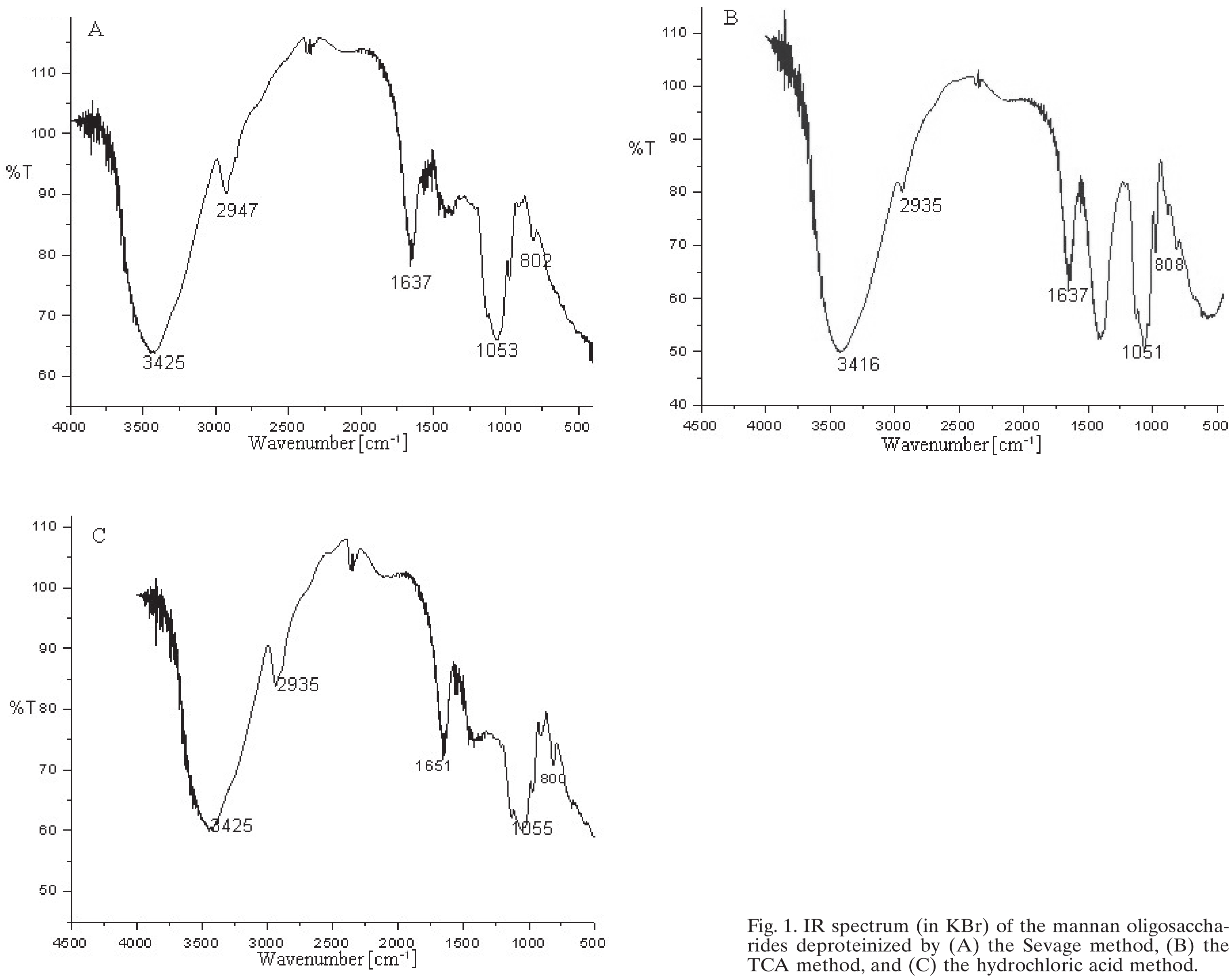

Fig. 1. IR spectrum (in KBr) of the mannan oligosaccharides deproteinized by (A) the Sevage method, (B) the TCA method, and (C) the hydrochloric acid method.

acid method is used for deproteinization because the solubility of a protein is the lowest at pI. The results are shown in Table I. They indicate that the hydrochloric acid method exhibits the highest percentage of deproteinization, but only a little higher percentage of mannan oligosaccharide loss than the other two methods. The Sevage reagent, which contains poisonous chloroform, is environmentally disadvantageous. There was more loss in the recovery of mannan oligosaccharides using the TCA method and hydrochloric acid method, which may be due to more evident damage of mannan oligosaccharides caused by TCA and hydrochloric acid, respectively.

Material and Methods

General

Yeast cell walls were purchased from Angel Yeast Co., Ltd. (Yichang, China). IR spectra were recorded with an FT-IR apparatus, and wavenumbers are reported in cm^{-1}. The concentration of proteins (c) was determined by UV absorption using the relationship: $c = 1.45A_{280} - 0.74A_{260}$, where A_{280} and A_{260} are the absorbances at 280 and 260 nm, respectively. This method will correct for any interfering absorbance due to nucleic acid present in the solution (Layne, 1957). The concentration of mannan oligosaccharides was determined by the phenol-sulfuric acid method using glucose as standard (Dubois *et al.*, 1956).

Extraction of crude mannan oligosaccharides

The water-soluble mannan oligosaccharides were obtained from 5 g yeast cell walls by extraction with 1% NaOH (50 mL) at 100 °C for 2 h, cooling and neutralizing to pH 7 with dilute HCl solution. After filtration, the mannan oligosaccharides were precipitated by adding 200 mL (4 volumes) of absolute ethanol. The precipitate was washed with absolute ethanol and diethyl ether, respectively.

Table I. Comparison of three techniques for deproteinization [a].

Method	Deproteinization (%)	Mannan oligosaccharide loss (%)
Sevage	89.8 ± 0.6	12.2 ± 0.4
TCA	91.4 ± 0.5	19.0 ± 1.0
Hydrochloric acid	96.7 ± 0.8	22.3 ± 1.7

[a] Values are the mean ± standard deviation of three separate determinations.

Deproteinization by the Sevage method

The concentrated solution of crude mannan oligosaccharides was combined with 0.2 volumes of chloroform/isoamyl alcohol (5:1 v/v) and vigorously shaken in a separatory funnel for 5 min. The aqueous phase was centrifuged at 2,000 rpm for 10 min, and the aqueous layer was carefully drawn off from the residual chloroform layer. The chloroform layer was discarded. This procedure was repeated 3–5 times until no further precipitate was observed at the interface. The mannan oligosaccharides were precipitated with 3–4 volumes of ethanol from the aqueous phase.

Deproteinization by the TCA method

The concentrated solution of crude mannan oligosaccharides was adjusted to pH 3 with 10% TCA solution overnight. The sample was centrifuged for 10 min at 5,000 rpm and the precipitate discarded to obtain the deproteinized solution. This procedure was repeated 1–2 times. The mannan oligosaccharides were precipitated according to the Sevage method.

Deproteinization by the hydrochloric acid method

The concentrated solution of crude mannan oligosaccharides was adjusted to pH 3 with 2 M hydrochloric acid overnight. The sample was centrifuged for 10 min at 5,000 rpm and the precipitate discarded to obtain the deproteinized solution. The mannan oligosaccharides were precipitated according to the Sevage method.

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