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Original Article

A Modified Method for Extraction and Deproteinization of Mannan Oligosaccharides

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ABSTRACT

Extraction of water soluble mannan oligosaccharides (MOS) from the cell wall of yeast and a modified method of deproteinization were studied. This method depends on the complete inactivation of residual proteins present after deproteinization by Sevage method (Staub 1965) using acetone. IR spectral analysis clearly indicated that the sample purified by modified Sevage method was found to be chemically mannan oligosaccharides. Proteins would have completely inactivated due to the breakdown of hydrogen bond by the electronegative oxygen atom present in the C=O group of acetone. The proposed method can be considered as an modified method of Sevage method. Sevage reagent contains poisonous chloroform which is environmentally disadvantageous (Huang et al., 2005) and the present method removes or inactivates the residual proteins and avoids the health and environmental hazards associated with chloroform by using it not more than once. Proteins and nucleic acids contamination were identified using UV spectra of the samples read between 200-600 nm. The concentration of MOS was carried out by Dubois method. In summary, we obtained highly purified MOS by the proposed method with low concentration of protein contaminants.

Key words: Yeast cell wall, Mannan Oligosaccharides, Extraction, Deproteinization.

INTRODUCTION

Yeasts are single celled fungi used for baking and production of alcoholic beverages for thousands of years. It is widely used in wine industry for its ability to bind undesirable components which allows it to prevent and cure stuck fermentation. Yeast cells are enclosed by a cell wall containing 30-60% polysaccharides (29-64% β glucan & 31%mannanoligosachharides)[1] 13% proteins, 9% lipids and 1-2% chitin. Usually proteins are linked to the mannan oligosaccharides (MOS) and are known as alkali soluble mannoprotein complex[2].

When MOS is included in the animal feed, it is used as diarrhoea preventative in weaning pigs by binding with pathogens in the intestinal wall thereby inhibiting bacteria colonization. MOS is also used as prebiotic serving as a nutrient source and stimulate the growth and activity of beneficial bacteria in the intestinal wall. In the present study, modified extraction and purification method have been studied to obtain more safe and pure alkali soluble MOS from the cell wall of yeast.

MATERIAL AND METHODS

The yeast material was purchased from Prakash food products, Bellur, Mysore. IR spectrum was recorded with an FT-IR spectroscopy and wavenumbers are reported in cm^{-1}. An UV absorbance ratio at A/260/280 is used to characterize if the MOS sample contained proteins and nucleic acids. The concentration of MOS in deproteinized aqueous solution was determined by phenol-sulphuric acid method using dextrose as standard[3].

Extraction of mannan oligosaccharides: [4] Air dried yeast (10 g) was steeped in 1% NaOH (70ml) and extracted for 2 hours at 100 ^oC to break and release mannan oligosaccharides from protein bonds. It is cooled and neutralized to pH 7 with dilute hydrochloric acid. Then the milky liquid was filtered through fine muslin with several washes and part of protein was removed during filtration. Equal volume of ethanol was added to the filtrate to precipitate the MOS and kept aside in a refrigerator for one day for effective setting. The precipitate was filtered and washed successively with 100 ml each of absolute ethanol and diethyl ether (twice)

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respectively. It is then dried completely at 37 ºC to obtain the crude MOS. UV spectrum of crude MOS was read between 200-600 nm.

Deproteinization of mannan oligosaccharides: Deproteinization of mannan oligosaccharide was carried out by using the Sevage method with minor modifications. In brief, partially purified MOS crystals were dissolved in double distilled water and treated with 50 ml of chloroform containing 5ml iso-amyl alcohol (Sevage reagent) in a separating funnel for 10 minutes. The aqueous layer was then centrifuged at 2000 rpm for 10 minutes as per Sevage method[5]. Since the sevage reagent contains poisonous chloroform, we have avoided the repeated use of chloroform. Instead, the MOS were precipitated with equal volume of ethanol from the aqueous phase. It was filtered, dried and powdered. Thus partially purified MOS crystals were dissolved in double distilled water and generally called as the aqueous extract of MOS. Known volume of aqueous MOS was treated with 3 volumes of ice cold acetone and kept at 4 ℃ for 2 hours to precipitate MOS and inactivate the residual proteins. It was filtered, dried and powdered. UV spectrum of deproteinized sample was read between 200-600 nm.

Quantification of Protein: The amount of protein present was carried out according to Bradford's method[6] using Bovine albumin (BSA) as standard. 20-100 µl of standard was taken in series of test tubes. 20 µl of the samples were taken in separate test tubes and the volume was made up to 100 µl with distilled water. 900 µl of the Bradford's reagent was added to these tubes and mixed well. Absorbance was read at 595 nm. The concentration of protein was calculated according to the standard calibration curve.

Estimation of Total sugars: The total sugar concentration was estimated according to Duboi's method. 200-1000 µl of the working standard solution (10-50 µg/ml) was pipetted into a series of test tubes. 25 µl of the samples were taken in separate test tubes and the volume was made up to 1ml with distilled water. To this 1ml of 5% phenol and 5 ml of concentrated 36 N sulphuric acid were added and mixed well carefully. Orange colour developed was read at 520 nm immediately. The total sugar concentration was calculated according to the standard glucose calibration curve.

RESULTS AND DISCUSSION

Mannan oligosaccharides (MOS) were extracted by dilute alkali method and partially purified or deproteinized by most widely used Sevage method with slight modifications. The principle involved in Sevage method is that the dissociative protein is denatured by sevage reagent to an insoluble substance and removed by filtration. The present modified sevage method avoids toxicity due to the interference of poisonous chloroform and it also helps to remove small quantity of protein from MOS. Proteins and nucleic acids absorb in the UV region at wavelength with absorbance maxima at 280 and 260 nm respectively. Nucleic acids show absorbance in the UV region because of the presence of purine and pyrimidine bases containing conjugated double bonds. Proteins have absorbance maxima at 280 nm due to the presence of aromatic amino acids. They also show absorbance at 260 nm but to a lesser extent. The UV absorbance spectra of aqueous extract of MOS before deproteinization shows absorbance at 280 nm. It is shown in figure 1. Minor amount of protein remained along with MOS was precipitated by acetone precipitation as shown in the Table 1. Compared to the aqueous extract, the concentration of the protein is more in acetone precipitated sample (0.072 g %). This indicates, that the acetone precipitation is the best method to remove the protein and to inactivate the protein content present in aqueous extract along with MOS. Due to the treatment of ice cold acetone, protein content is completely precipitated and it is in the inactive form wherein the hydrogen bond of the protein would have completely breakdown by the electronegative atom present in the C=O group of acetone. Hence there is no absorption at 260-280 nm which is the specific absorption for protein (Figure 2). The concentration of MOS was confirmed by the sugar estimation by Duboi's method. Amount of sugar in the acetone precipitated sample (5.56 g %) is almost equal to the amount of sugar present in the aqueous MOS (5.60 g %) It is shown in Table 2. The IR absorption at 3418 cm^{-1}indicated OH bands at lower frequency. The presence of carbonyl group bands at 1632 cm^{-1} proved to have the residual protein in mannan oligosaccharides sample and also it contains absorption bands arising from the v(CC) and v(COC) stretching vibrations at 808 assigned to the corresponding alpha mannosidic (C-H) stretch. IR spectral analysis clearly indicated that the sample purified by the present modified method was found to be chemically MOS.

CONCLUSION

Sevage reagent for deproteinization contains poisonous iso-amyl alcohol which is environmentally disadvantageous and more than one use cannot be preferred. In the present method, we obtained deproteinized MOS by safe and reliable method with low concentration of protein contaminant. In addition, research is continuing for further purification and pharmaceutical uses of MOS.

Figure 1.The UV absorbance spectra of aqueous extract before deproteinization

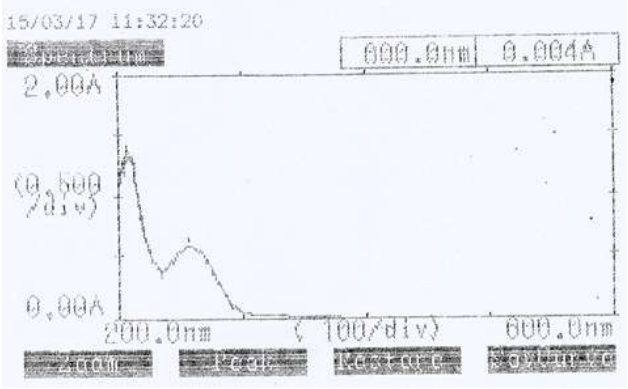

Figure 2. The UV absorbance spectra of MOS precipitate after deproteinization

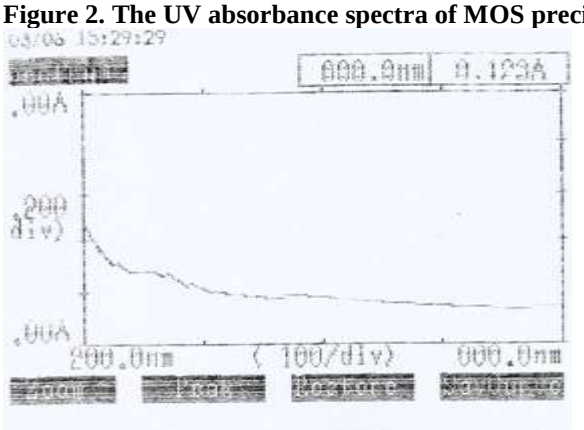

Table 1. Concentration of protein in aqueous extract and precipitate of MOS

Samples used	**Concentration of protein (in g%)**
Aqueous extract	0.048
Acetone precipitate of MOS	0.072

Table2.Concentration of sugar in aqueous extract and precipitate of MOS

Samples used	**Concentration of sugar (in g%)**
Aqueous extract	5.60
Acetone precipitate of MOS	5.56

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