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Analysis of Different Extraction Methods on the Yield and Recovery of β-Glucan from Baker's Yeast (*Saccharomyces Cerevisiae*)

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Abstract

β-glucans are complex, high molecular weight polysaccharides, found in the cell wall of many yeasts and cereals. Yeast β-glucans differ from their cereal counterparts, as they comprise a mixture of β-1,3 and 1,6 glucans compared to the cereal derivatives which are a mixture of β-1,3 and 1,4 glucans. Immuno-stimulation is the most important property of β-glucan. β-glucan, both *in vitro* and *in vivo,* inhibits cancer cell growth, prevents or reduces bacterial infection. Present study focused on the different isolation methods to extract β-glucan from the selected three different commercial Baker's yeasts. The β-glucan yield and recovery varied from 17.9% to 49.2% and 18.5% to 86.3% from the three different methods. The results of the present study indicate that the highest β-glucan yield was obtained from enzymatic isolation method has a potential to be used as a functional ingredient in foods and feeds.

Keywords: β-glucans, Immuno-stimulation, *Saccharomyces cerevisiae* etc.

1. Introduction

β-glucans are polysaccharides whose glucose units are linked with β-1,3 glycosidic bond (Freimund et al., 2003) [1]. β-glucans are found in sources like bacteria, ffungi, yeasts, algae and higher plants, mainly cereals (oats and barley). In cereals the basic units of glucose chains in β-glucans are inter-linked by β-1,3 and β-1,4 bonds (Velisek., 1999) [2] whereas in microorganisms β-glucans are formed by a linear central backbone of D-glucose linked in the β-1,3 position with glucose side branches β-l,6 of various sizes (Mantovani et al., 2008) [3]. One of the important sources of β-glucans are cell wall of yeast (*Saccharomyces cerevisiae*) especially the baker's and brewer's yeast. In Saccharomyces cerevisiae, the main structural part of the cell wall are β-1,3 glucans consisting of 30-45% by weight of the cell wall and β-1,6 glucan of 5-10% which is relatively small but it is very important for cross linking (Soares and Soares., 2011) [4].

Generally, yeast has been used for baking and ethonal production for thousands of years. Yeast β-glucans are attracting increasing attention in the pharmaceutical and food industry for its beneficial effects on human health. Researches attribute β-glucan derived from yeast has multilateral biological activity. β-glucan derived from yeasts are water insoluble (Santipanichwong and Suphantharika., 2009) [5]. The Insoluble glucans may be converted to water soluble form through chemical modification (D.L. Williams., 1992) [6].

β-glucan extraction from *Saccharomyces cerevisiae* yeast cell consists of two stages: 1. Yeast cell lysis: β-glucan is localized in cell wall, thus it is necessary to lyse cells and separate insoluble cell wall from cytoplasm; 2. β-glucan extraction from insoluble cell wall. There are several groups of yeast lysation methods – chemical, physical and enzymatic. Chemically, yeast cells are lysed by Sodiumhydroxide, Hydrochloric acid, acetic acid, citric acid and using chemically aggressive solutions. In most cases chemical lysis is held at high temperatures (boiling) (Pelizon et al., 2005 [7]; Hunter et al., 2004 [8]; Jamas et al., 1989 [9]; Lee et al., 2001 [10]). Physically yeasts are disrupted using sonication, homogenizers with high pressure (Shokri et al., 2008 [11]; Boonraeng et al., 2000 [12]; Wenger et al., 2008 [13]). In Enzymatic methods used for yeast degradation, cell lysis is done using natural enzymes. Enzymes partially destroy yeast cell wall, so that soluble cytoplasm leaks to the surface.

The aim of this present work is to isolate β-glucan from three different methods in-order to determine the maximum recovery of β-glucan content from *Saccharomyces cerevisiae.*

2. Materials and Methods

2.1 Identification of Yeast

Yeast, of various forms and types are commercially utilized - Creamyeast, Fresh yeast, Dry yeast, Semi-dry yeast and Active dry yeast. Among them Active dry yeast offers excellent stability at ambient temperature with longer shelf life. Hence in the present study commercially available active dry yeasts was selected for the analysis.

2.2 Procurement of Raw Material

Commercially available dried baker's yeasts of three different brands were selected (Brand I, II and III) for β-glucan isolation. This was procured from the local market, puducherry region.
Yeast β-glucan Assay kit was obtained from Megazyme, Ireland. All other chemicals used in this study were of analytical grade procured from Himedia, Chennai.

2.3 Determination of β-glucan Content

Glucan contents in yeast powder were analyzed using Yeast β-glucan Assay Kit (Megazyme, Ireland). For β-glucan content analysis, 100 mg of yeast cell were used. It was calculated as the difference between total glucan and alpha glucan.
β-glucan (%) (w/w) = [(total glucan oligomers) – (alpha glucan oligomers)]

2.4 Extraction of β-glucan

From the above result of the β-glucan estimation, one yeast brand was selected and utilized for the extraction procedure. Three different extraction methods adopted are as follows.

Method 1 (Alkali method): β-glucan was isolated by a modified method of Williams et al., 1991 [14].

Method 2 (Alkali-acid method): A combination method of Williams et al., 1991 [14] and Hunter et al., 2004 [8] was adopted.

Method 3 (Enzymatic method): Combination method of Freimund et al., 2003 [1]; Pornchalearn., 2006 [15]; Liu et al., 2008 [16] with slight modification was followed for the β-glucan extraction.

2.5 Identification of Yield and Recovery of β-Glucan in the Crude β-Glucan Extract

The extracted crude β-glucan powder was tested for its yield by weighing the dried extract powder. The recovery of β-glucan content was determined by estimating total β-glucan content in the extracted powder as said above with the yeast β-glucan assay kit (Megazyme, Ireland) and calculated as the percentage ratio of weight of β-glucan in crude extract to the weight of β-glucan present in 100gm of flour.

3. Results and Discussion

3.1 Selection of Commercial Yeast

Among the different brands of active dry yeast, three selective brands (Brand I, II and III) were found to be most preferably procured and utilized by the customers with maximum turnover. Hence for the estimation of β-glucan these three brands were selected.

3.2 Estimation of β-glucan content in Baker's yeast

It is well known that β-glucans have several beneficial properties and potential use in food, chemical and pharmaceutical industries. Baker's yeast is the best source of β-glucan. Seeley (1977) [17] described the utilization of proteins, yeast extract and β-glucan isolated from baker's yeast in food industry (D. G. Hayen., 2001) [18].

Table 1: Percentage of β-glucan Content

Baker's yeast (Saccharomyces Cerevisiae)	***β-glucan (%) (w/w)***
Brand I	1.96
Brand II	7.35
Brand III	0.34

The experiment conducted on 3 different branded active dry yeasts (S. Cerevisiae) revealed that the maximum percentage of β-glucan content was identified in Brand II (7.35%) followed by brand I (1.96%) and III (0.35%). The difference in the content of β-glucan in each brand may be due to the difference in the Yeast strain.

3.3 Estimation of crude extract of β-glucan from Baker's yeast

The extraction of baker's yeast by different methods produces wet β-glucan extract of varying weights. When the obtained bright yellow product is vaccum dried, it turns to white to pale yellow powder. The percentage of crude β-glucan extract from 60 grams of baker's yeast are given in the below table

Table 2: Percentage of Crude β-glucan Extract

Methods	***Weight of Baker's Yeast (gm)***	***Weight of Extract (gm)***	***Percentage of Extract (%)***
Alkaline	60	11.6	19.3
Alkaline-acidic	60	29.5	49.2
Enzyme	60	10.7	17.9

The present study proves that the yield of crude β-glucan extract varies from 17.9% to 49.2% depending on the isolation procedure. Maximum yield (49.2%) was obtained in the alkaline acidic method. Many authors reported varying yield of extraction 11.84% and 8.08% by alkaline acidic and alkaline acid isolation with mannoprotein removal (Vesna Zechner et al., 2010) [19], 12.3% extraction yield by alkaline acidic method (Ali. J. Jabber et al., 2011) [20].

3.4 Estimation of β-glucan content from the crude β-glucan extract

The isolated crude β-glucan extract from different methods of varying yields was estimated to determine the content of β-glucan. Further to identify the efficiency of various extraction methods, β-glucan recovery was calculated. β-glucan recovery corresponds to the percentage ratio of weight of β-glucan in crude extract (that was obtained from 60gm flour) to the weight of β-glucan actually present in 100gm flour and it shows the efficiency of purification process of β-glucan.

Table 3: Percentage of Recovery of β-glucan content

Methods	*β-glucan (%) (w/w)*	*Percentage of Recovery (%)*
I	1.36	18.5
II	1.74	23.7
III	6.34	86.3

The efficiency of different extraction methods on the β-glucan recovery showed in the above table (3). Among the three different methods, enzymatic method of extraction along with the physical lysis (sonication) revealed maximum recovery of β-glucan. 6.34% of β-glucan content with 86% of recovery shows closeness to the original content of β-glucan in the active dry yeast. The present study slightly coordinates with the study conducted by chemma borchani et al., 2014 [21] that showed enzymatic process for fractionation of baker's yeast cell wall completely recovered the content of β-glucan (11.8%) present in the original ratio in the cell wall of yeast.

4. Conclusion

In the present paper, different isolation methods have been adopted to produce valuable β-glucan from baker's yeast. The choice of yeast isolation process appeared to be important as it affect the most important property – the total β-glucan content recovery. Enzymatic treatment along with physical lysis by sonication was found to be of good choice compared to the other methods, since it gave highest recovery of β-glucan content. In conclusion, the isolation process used in this study can be utilized to produce β-glucan from *Saccharomyces cerevisiae* in large scale which has great potential in food industry.

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