International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 6(4): July-August 2016

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences

Research Article……!!!

Received: 15-06-2016; Revised: 02-07-2016; Accepted: 03-07-2016

EXTRACTION OF β GLUCAN UNDER ALKALI CONDITION AND FINDING OUT THE OPTIMUM CONCENTRATION OF ALKALI FOR EXTRACTION

Subhakankha Manna*, Supriya Kale

Department of Biotechnology, R. Ruia College, Matunga, Mumbai 400 019.

Keywords:

β-glucans, extraction, Saccharomyces cerevisiae

For Correspondence:

Subhakankha Manna

Department of Biotechnology, R. Ruia College, Matunga, Mumbai 400 019

E-mail:

subhakankha@gmail.com

ABSTRACT

β-glucans are complex, high molecular weight polysaccharides, found in the cell wall of many yeasts and cereals like oats. Yeast β-glucans comprise a mixture of β-1,3 and 1,6 glucans as 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 (Chen et al., 2007). Determination of optimum concentration of alkali for extraction of β-glucan from *Saccharomyces cerevisiae* is necessary to get maximum yield. The result shows that the highest β-glucan yield was obtained when lysed under 0.2N NaOH.

INTRODUCTION

The oat (*Avena sativa*), sometimes called the common oat, is a species of cereal grain. Oats are grown in temperate regions. They have a lower summer heat requirement and greater tolerance of rain than other cereals, such as wheat, rye or barley, so are particularly important in areas with cool, wet summers, such as Northwest Europe and even Iceland. Oats are an annual plant, and can be planted either in autumn (for late summer harvest) or in the spring (for early autumn harvest). Yeasts (Saccharomyces) are eukaryotic microorganisms classified as members of the fungus kingdom with 1,500 species currently identified and are estimated to constitute 1% of all described fungal species. Yeasts are unicellular, although some species may also develop multicellular characteristics. Beta glucans can be extracted from both oats and yeast.

Glucans-Many studies have shown the beneficial health effects of oats. Consuming oats lowers the levels of blood cholesterol and attenuates postprandial glucose response (Braaten et al., 1994; Wood et al., 1994a, b; Wood et al., 2000). This is believed to be caused by the main component of soluble dietary fibre of oats called (1→3),(1→4)-β-D-glucan also referred to as β-glucan (Ripsin et al., 1992; Tappy et al., 1996). The positive health effects are believed to be caused by the viscosity of β-glucan (Guillon, Wood, 2004). Viscosity in turn is affected by concentration and molar mass. Consequently, these factors must be taken into account when health effects are to be considered. Therefore extractability and effects that processing may have on are important.

Types of Glucans:

α-Glucan:Polysaccharides of D-glucose monomers linked with glycosidic bonds of the alpha form. *β-Glucan* is a polysaccharide. It consists of repeating units of D Glucose monomers linked by β glycosidic bonds. β-glucan is found is cell wall of *Saccharomycescerevisiae,* oats and barley.β-Glucan are of two types β(1-3) for a glycosidic linkage indicates that the etheric oxygen bridge between two consecutive monosaccharide units of the polysaccharide connects the number 1 carbon of the first unit to the number 3 carbon of the second unit, and that etheric oxygen bridge attaches to carbon 1 of the first unit from above the ring. Likewise, the designation of β-(1,6) for a glycosidic linkage indicates that the etheric oxygen bridge between two consecutive monosaccharide units of the polysaccharide connects the number 1 carbon of the first unit to the number 6 carbon of the second unit, and that etheric oxygen bridge attaches to carbon 1 of the first unit from above the ring.

The β-(1,3) glucan chains with a degree of polymerization of ~1500 glucose units/chain has a coiled spring like structure that provides high elasticity and tensile strength to the cell wall. In cell wall extracts, β-(1,3) glucan is found as a branched polymer with β-(1,6) interlinked chains. In contrast to the microfibrillar structure of β-(1,3) glucan in *Saccharomycescerevisiae* this β-(1,6)polymer is shorter than β-(1,3)glucan is amorphous structure acts as a flexible glue forming covalent cross links to β-(1,3)glucan and chitin to the cell wall mannoproteins.

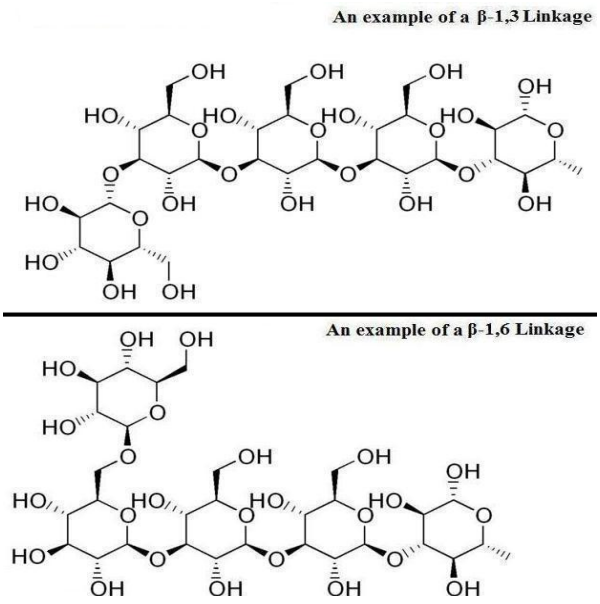

Image 1- 1,3 and 1,6 linkagesseperately

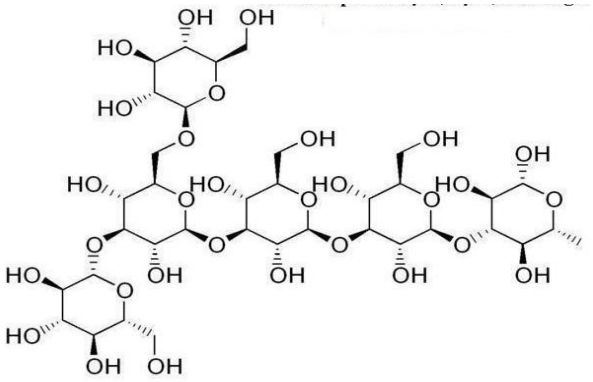

Image 2- β-1,3; β-1,6-LINKAGE together

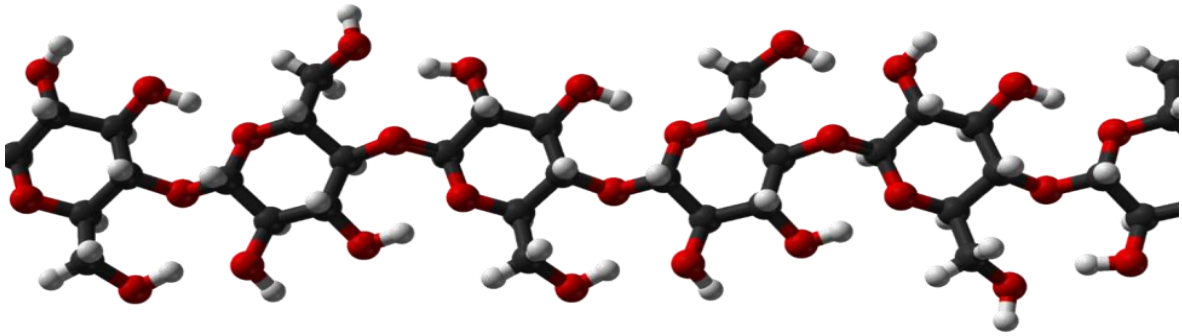

Image 3- β-1,3; β-1,6-LINKAGE together

Applications of β –glucan: Nowadays the fungal β glucan are broadly investigated as they are recognized as potent immunological stimulators for human immune system, β glucan also protect mammals from different infections and increases the immune cells cytotoxicity against cancer cells (Chen et al., 2007). These are the main reasons for the extraction of the β glucan.

DIFFERENT METHODS OF EXTRACTION

There are several groups of yeast lysation methods – chemical, physical and enzymatic. Chemically, yeast cells are lysed by NaOH or any other alkali.

Physically yeasts are disrupted using sonication, homogenizers with high pressure.

In Enzymatic method, Enzymes partially destroy yeast cell wall, so that soluble cytoplasm leaks to the surface.

The method used here is chemical method based on *Saccharomyces cerevisiae* yeast cell digestion by using alkaline solution of various concentrations. Standard solutions of NaOH were prepared to lyse the yeast cell and to check the maximum extraction concentration of NaOH.

Extraction of β glucan from *Saccharomyces cerevisiae* consists of two steps:

1. Cell lysis: Separating insoluble cell wall from the cytoplasm
2. B glucan separation from the insoluble cell wall

Alkaline lysis was first described by Birnboim and Doly in 1979 (Nucleic Acids Res. 7, 1513-1523). Alkaline lysis of cell is a method to disrupt the cell wall, under alkaline conditions the ester, non-covalent and covalent bonds are broken and polysaccharides are released from the complex network of cell. The alkali chosen is different concentrations of sodium hydroxide in order to determine the best concentration for cell lysis. Acid hydrolysis is also used in lysating cells, however alkaline hydrolysis was chosen because under acidic conditions the glycosidic linkages have a high chance of getting hydrolyzed, which may result in breaking of the glucan structure.

MATERIALS AND METHODS

Media Preparation and Culturing of yeast cells - *Saccharomyces cerevisiae* was cultured by inoculation of 0.1 ml of culture into 100ml Sabourauds dextrose broth and was incubated for 48hours at RT for maximum cell growth.

Extraction of β glucan -The culture was divided into 5 batches of 10 ml each, each batch was centrifuged for 30 minutes to separate the cells from the media, the supernatant (media) was discarded and each batch was added with 5 ml of NaOH of a specific concentration which was

prepared earlier, mixed, centrifuged for 30 minutes which created alkali conditions, resulted in the separation of cell wall from the remaining cell components. The residue was discarded this time. The supernatant containing β glucan which is a part of cell wall was estimated using cellulose estimation method.

β glucan Assayby cellulose estimation method - Acetic/Nitric was prepared using mixing 150ml of 80%Acetic acid and 15ml of conc. H_2SO_4in a clean 250ml flask, Anthrone reagent was prepared by adding 200mg of Anthrone powder to 100ml of concH_2SO_4, 67% H_2SO_4 was prepared from 100% concentrated H_2SO_4 stock.

The steps followed in estimation were:

1. Add 1ml of the supernatant containing β glucan with 3 ml of acetic/nitric reagent and mix in vortex.
2. Keep it in water bath for 30 minutes followed by cooling and centrifugation for 20 minutes.
3. Add 5 ml 67% H_2SO_4 and leave it for 1 hour.
4. Take 1 ml of above solution and add 10ml of anthrone reagent
5. Heat the tubes in water bath and check absorbance at 630nm.
6. Blank preparation by adding 1 ml of distilled water to 10 ml Anthrone reagent.

RESULTS AND DISCUSSIONS

Extraction of β-glucan was carried out in alkaline conditions by using NaOH of different concentrations, under alkaline conditions the ester, non-covalent and covalent bonds are broken and polysaccharides are released from the complex network of cell. Alkaline extraction was chosen over acidic extraction because under acidic conditions the glycosidic linkages have a high chance of getting hydrolyzed. Estimation of β glucan was carried out by cellulose estimation method by use of acetic/nitric reagent, Anthrone reagent and 67% H_2SO_4, this estimation method was chosen because of the structural similarity between cellulose and β glucan. Modifications were made to the standard cellulose estimation method (Updegroff, D M (1969) *Anal Biochem* 32 420), in this case as the amount of sample used was less, so the reagents were used accordingly. The graph of absorbance vs. concentration was plotted and it was observed that the highest absorbance reading was obtained for 0.2N NaOH solution. β glucan are broadly investigated as they are recognized as potent immunological stimulators for human immune system, many research professionals have already conducted research on β glucan, as they have wide range of applications in field of medicinal biology. Some of them are enlisted below.

1. The protective effect of beta-glucan against oxidative injury caused by acetaminophen was studied in mice liver. Acetaminophen caused a significant decrease in the GSH (*glutathione)* level of the tissue, beta glucan treatment reversed all of these [liver toxicity] biochemical indices, as well as histopathological alterations that were induced by acetaminophen. In conclusion, these results suggest that beta-d-glucan exerts cytoprotective effects against oxidative injury through its antioxidant properties and may be of therapeutic use in preventing acetaminophen toxicity (Toklu HZ, Sehirili AO)
2. Beta-glucans have been reported to function as a potent adjuvant to stimulate innate and adaptive immune responses, Treatment of orally administered yeast-derived particulate B-glucan elicited potent antitumor immune responses and drastically down-regulated immunosuppressive cells, leading to the delayed tumor progression (Qi C, Cai Y, Ding, Li B, Kloecker G, Qian K)
3. The significant role of glucans in cancer treatment, infection immunity, stress reduction and restoration of damaged bone marrow has already been established.(Vetvicka V)
4. The soluble phosphorylated glucans are useful for stimulating macrophage cells, either in vivo or in vitro, to produce a cytotoxic/cyctostatic factor effective against cancer cells (Williams D.L., Browder I. and DiLuzio N.R)
5. The protective effect of beta-glucan against oxidative injury caused by acetaminophen was studied in mice liver. Acetaminophen caused a significant decrease in the GSH (*glutathione)* level of the tissue, beta glucan treatment reversed all of these [liver toxicity] biochemical indices, as well as histopathological alterations that were induced by acetaminophen. In conclusion, these results suggest that beta-d-glucan exerts cytoprotective effects against oxidative injury through its antioxidant properties and may be of therapeutic use in preventing acetaminophen toxicity (Toklu HZ, Sehirili AO)
6. Beta-glucans have been reported to function as a potent adjuvant to stimulate innate and adaptive immune responses, Treatment of orally administered yeast-derived particulate B-glucan elicited potent antitumor immune responses and drastically down-regulated immunosuppressive cells, leading to the delayed tumor progression (Qi C, Cai Y, Ding, Li B, Kloecker G, Qian K)
7. The significant role of glucans in cancer treatment, infection immunity, stress reduction and restoration of damaged bone marrow has already been established.(Vetvicka V)

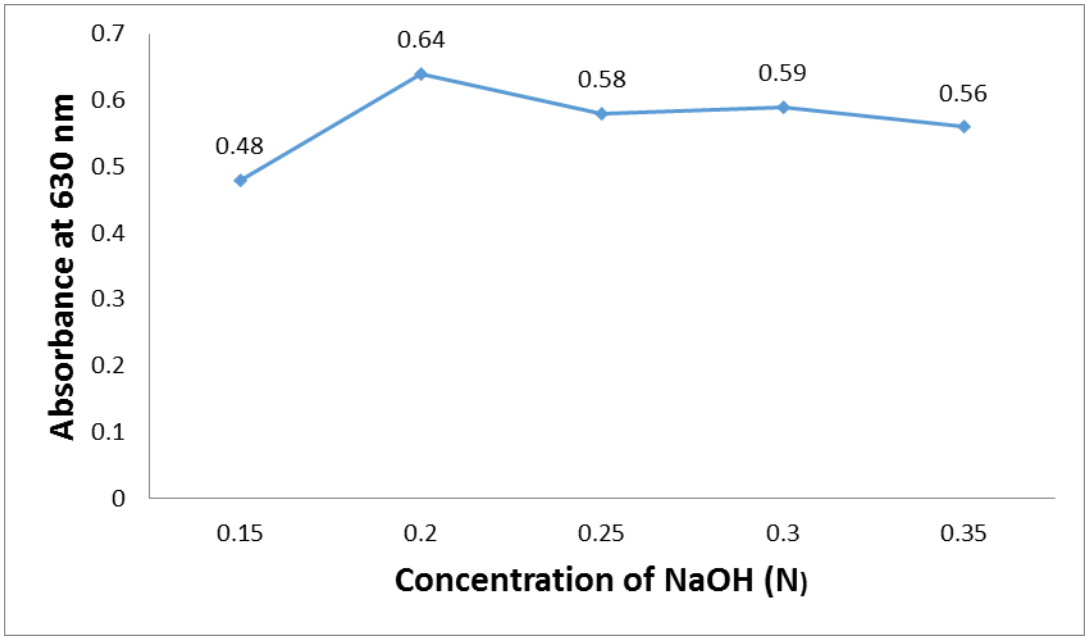

Image 4 - GRAPH OF ABSORBANCE AT 630 nm VS CONCENTRATION OF NaOH

CONCLUSION

Finding out the optimum concentration of NaOH for the maximum yield of β glucan becomes important as studies on β-glucan indicates that it has vast applications in biological sciences. β-glucan is a potent immunological stimulator for human immune system (Chen et al., 2007). Experimental data show that β-glucans protect mammals from different infections and increase immune system cells cytotoxicity against cancer (Vetvicka, 2011; Chen et al., 2009), it also has a role in diagnostic technique to detect fungal infections and it has been effective in lowering cholesterol levels.

REFERENCES

1. Artūras Javmen*, Saulius Grigiškis, Raimonda Gliebutė (2012) β-glucan extraction from *Saccharomyces cerevisiae* yeast using *Actinomyces rutgersensis* 88 yeast lyzing enzymatic complex. BIOLOGIJA Vol. 58. No. 2. P. 51–59.
2. Braaten JT, Wood PJ, Scott FW, Wolynetz MS, Lowe MK, Bradley-White P, Collins MW. (1994) Oat β-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. Eur J ClinNutr 48: 465-474.
3. Chen et al (2009) The effects of β-glucan on human immune and cancer cells. J HematolOncol. 2009; 2: 25. doi: 10.1186/1756-8722-2-25.
4. Guillaume Lesage and Howard Bussey (2006) Cell Wall Assembly in *Saccharomyces cerevisiae*" Microbiol Mol Biol Rev 70(2): 317–343.
5. H.C. Bimboim and J. Doly(1979), "A rapid alkaline extraction procedure for screening recombinant plasmid DNA"Nucleic Acids Res 7(6): 1513–1523.

6. Qi C, Cai Y, Ding, Li B, Kloecker G, Qian K, Vasilakos J, Saijo S, Iwakura Y, Yannelli JR, Yan J; (2011) "Differential pathways regulating innate and adaptive antitumor immune responses by particulate." Blood 117(25):6825-36. doi: 10.1182/blood-2011-02-339812.
7. Ripsin CM, Keenan JM, Jacobs DR, Elmer PJ, Welch RR, Van Horn L et al. (1992) Oat products and lipid lowering. A meta-analysis. JAMA 267: 3317- 3325.
8. Tappy L, Gügolz E, Würsch P. (1996) Effects of breakfast cereals containing various amounts of β-glucan fibers on plasma glucose and insulin responses in NIDDM subjects. Diabetes Care 19: 831-834.
9. Toklu HZ, Sehirili AO, Velioglu-Ogunc A, Centinel S, Sener G; (2006) "Acetaminophen-induced toxicity is prevented by beta-d-glucan treatment in mice." European J Pharmacology; 543(1-3):133-40.
10. Updegroff, D M (1969) *Anal Biochem* 32 420.
11. Vetvicka, V; Dvorak B; Vetvickova J; Richter J; Krizan J; Sima P; Yvin JC (2007). "Orally administered marine (1-->3)-beta-D-glucan Phycarine stimulates both humoral and cellular immunity". International journal of biological macromolecules (England: Butterworth-Heinemann) 40 (4): 291–298.
12. Vetvicka V (2010)"Glucan-immunostimulant, adjuvant, potential drug," World J ClinOncol, 2(2):115-119.
13. Wood PJ. (1993) Physicochemical characteristics and physiological properties of oat (1→3),(1→4)-β-D-glucan. In Oat bran. Peter J. Wood (ed.), AACC, St Paul, MN, p. 83-112.
14. Wood PJ, Beer MU, Butler G. (2000) Evaluation of role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. Br J Nutr 84: 19-23.