Application of different methods for the extraction of yeast *β*-glucan

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Abstract

β-D-glucan is a D-glucose biopolymer. The cell wall of yeast contains *β*-D- glucans. *β*-Glucan has been isolated from barley, oat, mushrooms, algae bacteria and yeasts. Spent brewer's yeast has been widely used for *β*-glucan production but yeast waste biomass from wine industry has not been yet utilized. Yeast *β*-glucan can be incorporated in various functional food, beverage and pharmaceutical products. In recent years, there is an increasing interest arrived from the food and pharmaceutical research community due to its proven immunostimulating role in human and animal health. For the production and purification of yeast *β*-glucan various technologies have been proposed and new techniques are constantly investigated. The aim of our research was the study of three different methods and technologies for the extraction and purification of *β*-D-glucans from Vin 13, a *S.cerevisiae* strain used widely for wine production. The max. concentration (64.56 ± 1.25 %) of *β*-glucan in the final yeast powder was achieved with the extraction Method 3 in only two steps process and more precisely with 10% (w/v) yeast cell rehydration, 24 h yeast autolysis and 0.5 M hot NaOH. The demonstrated method is easy, fast and low cost for yeast *β*-glucan production in industrial scale.

Keywords: yeast *β*-D-glucans, *Saccharomyces cerevisiae*, extraction methods, winery waste

1. Introduction

Yeast glucans are polysaccharides that constitute structurally different D-glucose polymers and according to glucose anomeric structure they are distinguished to *α*-D-glucans, *β*-D-glucans and *α,β*-D-glucans (Synytsya et al., 2013). *β*-D-Glucan biopolymer is located in the cell wall of various organisms like yeast, bacteria, fungi, algae and plants. In yeast, it is a major component of cell wall (ca. 60% of cell wall dry mass) while the yeast cell wall accounts up to 30% of cell dry mass (Aguilar-Uscanga et al., 2003). In the yeast cell wall of *Saccharomyces cerevisiae*, linear (pachyman, pustulan) and branched (1→3),(1→6)-*β*-D-glucans (yeast wall glucan) are found (Stone, 2009; Kim et al., 2000). In yeast cell wall two different types of *β*-D-glucans are found: *β*-1,3-D-glucan of about 1500 residues which is the major component (85%) and represents more than 50-55% of cell wall and *β*-1,6-D-glucan of about 140 residues which is found in minor amounts (15%) (Lesage et al., 2006). Yeast *β*-glucan has been proved beneficial for the human and animal health system as it acts as an immunostimulator (Stier et al., 2014; Sze et al., 2012; Mantovani et al., 2008; Volman et al., 2008). For that reason, it belongs to the BRMs (Biological response Modifiers) and can be incorporated in functional food and medicines (Ahmad et al., 2012;

Sobieralski et al., 2012; Novak et al., 2008; Williams et al., 1992). *β*-Glucan can be isolated from the cell wall of baker's and brewer's yeast *S. cerevisiae* (Many et al., 2014). For the extraction and purification of yeast *β*-glucans, a significant number of different methods and technologies have been proposed and many of them have been patented (Kwiatkowski et al., 2012). The first *β*-glucan extraction methods were based on alkali-acid hydrolysis methods and later on cell oxidation by sodium hypochloride. In recent years, biotechnological isolation methods with enzymes and/or sonication treatment have been developed (Varelas et al., 2015; Many et al., 2014; Tam et al., 2013). The critical steps in the extraction process are the yeast cell lysis and subsequent the cell wall purification which must lead to the less degraded part of glucose chains, the distribution of *β*-glucan mass in the supernatant and thus to reduced yield (Varelas et al., 2015). Last years, the yeast waste biomass from brewery industry has been treated for *β*-glucan production (Pinto et al., 2015; Araújo et al., 2014; Thamakiti et al., 2004) but the spent yeast from winemaking process has not been yet utilized (Varelas et al., 2015). In this study, three different methods for the extraction of *β*-glucans from the cell wall of *S. cerevisiae* VIN 13 strain used widely in winemaking fermentations, were developed and compared in laboratory scale experiments. The purpose of this comparative study was the investigation and assessment of some of the available techniques for *β*-glucan production. The final scope of this study was the choice of a new easy, rapid and low cost method which can be used for yeast *β*-glucan production in industrial scale for the recovery of *β*-glucan from winery wastes (Nerantzis et al., 2006; Shrikhande et al., 2000).

2. Materials and Methods

2.1. Materials

Yeast cells VIN 13 *S. cerevisiae* strain were provided by Anchor. Enzymes *β*-glucanase (Glucanex 200 G), lipase (Lipopan Extra) and protease (Protamex) were provided from Novozymes, Denmark. NaCl, NaOH, isopropanol and all reagents used were of analytical grade

2.2. Methods

2.2.1. Extraction of (1,3)(1,6)-β-D-glucan: Method 1

β-Glucan was isolated from yeast cell walls by a modification of the method of Javmen et al., 2012 (Fig. 1).

2.2.1.1. Starting material-Yeast cells preparation

30 g of dry yeast VIN 13 were divided in three equal portions (each of 10 g) and then diluted to dionized water leading to yeast cells slurry of 10% (w/v). This was done according to manufacturer's instructions for the appropriate rehydration of yeast cells.

2.2.1.2. Yeast cells enzyme treatment/Yeast cell lysis

Yeast cells were treated with lytic enzyme Glucanex®200G (Novozymes, Denmark) for the cell wall disruption. The enzyme conditions were pH 4.64 and t=50^{o} C (Prieto et al., 2012). For the estimation of the appropriate amount of the enzyme for the cell wall disruption, three different concentration of the enzyme at 1, 10 and 50 times higher than the normal dose (0.015 g/l), were tested (Kim et al., 2006).

2.2.1.3. Extraction and isolation of yeast β-glucan

For the isolation of *β*-glucans from the yeast cell wall, NaOH in two different concentrations (0.5 and 1.0 M) was used. The yeast material produced after enzyme

treatment step was diluted in NaOH/90ºC/2h with stirring. The *β*-glucan sediment was obtained after centrifugation at 5000 rpm/4ºC/10min while supernatant was discarded.

2.2.1.4. Lyophilization
The sediment of *β*-glucans was frozen (-80ºC/24 h) and then lyophilized (-50ºC /vacuum/24 h) using a Thermo Fischer (USA) drying digital unit.

2.2.1.5. β-Glucan production
With extraction Method 1 four different insoluble *β*-glucans were produced and named respectively g-1, g-2 g-3 and g-4 (Table 1).

2.2.1.6. Yield calculation of produced β-glucan
The yield of produced *β*-glucan was calculated as the product of solids yield with *β*-glucan purity. The solids yield was calculated as the percentage of dry weight of crude *β*-glucan mixture from dry weight of the yeast cells starting material. The dry weight of crude *β*-glucan mixture was determined gravimetrically.

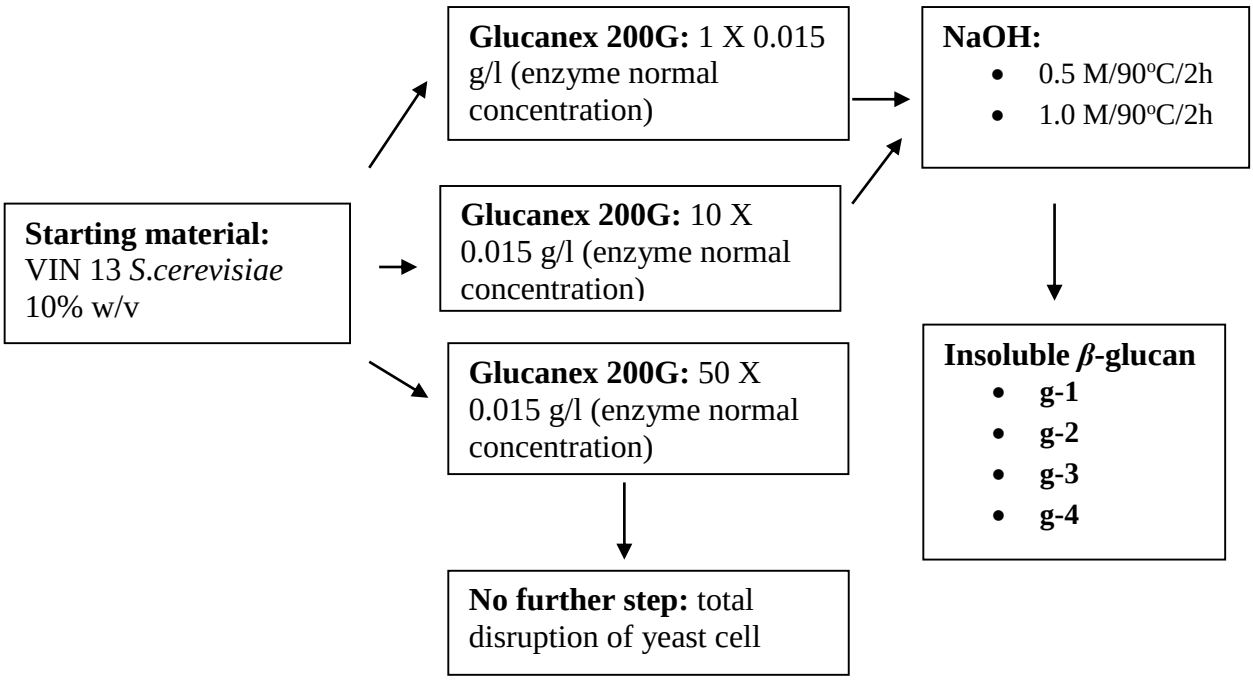

Fig. 1. Schematic diagram showing the process for the extraction of (1,3)(1,6)-*β*-D-glucan with extraction Method 1

2.2.1.7 Statistical analysis
All the experiments were repeated in triplicate. The values represent the average value of all replications with error bars representing standard errors of the average value of all replications with each range of determined parameter. In order to determine if significant differences existed between the different treatments and the tested parameters, all results were analyzed using one-way analysis of variance (ANOVA) run on XLSTAT software (Addinsoft Co.,USA). Significant differences were indicated at $P<0.05$.

2.2.2. Extraction of (1,3)(1,6)-β-D-glucan: Method 2
β-Glucan was isolated from yeast cell walls by a modification of the method of Magnani et al., 2009 (Fig. 2).

2.2.2.1. Induced autolysis
20 g of dry yeast VIN 13 were divided in two equal portions (each of 10 g) and diluted to deionized water leading to yeast cells slurry of 10% and 20% (w/v) respectively. The pH was brought to 5.0 with HCl 0.1 N and 3% NaCl was added as the autolysis promoter. The mixture was incubated at 55ºC for 24 h with mild agitation. Then, the autolysate was heated at 80ºC for 15 min for the deactivation of the endolytic enzymes. The yeast extract (supernatant) was separated from autolyzed yeast cells (sediment) with centrifuging (5000 rpm/10 min). The autolysis ratio was calculated as follows: 5.0 ml from the initial yeast slurry and 5.0 ml from the autolysate were dried and the dried biomass was weighted. The autolysis ratio (R) is the loss of dried biomass before (Wo, initial dried biomass in 5.0 ml suspension sample) and after autolysis (W, residual dried biomass in 5.0 ml suspension) (Liu et al., 2008; Yajun et al., 2003).
R(%)= [(Wo-W)/Wo] x 100

2.2.2.2. Hot water treatment
10% w/v in solid content of the sediment received after yeast autolysis was resuspended in 250 ml 0.02 M sodium phosphate buffer/pH 7.5 with glass beads (diameter 0.3-0.4 cm), heated to 121ºC in an autoclave for 4 h and then cooled down to 40ºC (Liu et al., 2008). The residue was separated from the supernatant with centrifuging (5000 rpm/10 min) for further use.

2.2.2.3. Sonication
Yeast cell material after hot water treatment was treated with ultrasounds for the further cell disruption. The sonication conducted at 35 kHz for 6 min (u=230 V/AC, I=1,6 A, f=50/60 Hz) in an ice bath with a Transsonic 570/H sonicator (Elma, Germany).

2.2.2.4. Lipid removal
The yeast cell material obtained after sonication was treated with organic solvent and lipase enzyme for the extraction of remained lipids. As an organic solvent, isopropanol was used in a yeast suspension: organic solvent of 1:4 (w/v). The suspension was heated under reflux for 2h. As a lipase, Lipopane Extra (Novozymes, Denmark), 0.5 U per gram of yeast material (5 h/pH 7.5/55ºC) under reflux, was used. In both cases, the suspension was cooled down to 28ºC and centrifuged (5000 rpm/10 min). Then, the recovered residue was washed three times with acetone 1:1 and centrifuged (500 rpm/5 min) (Magnani et al., 2009; Liu et al., 2008).

2.2.2.5. Protein removal
For the removal of proteins, protease Protamex (Novozymes, Denmark), 0.5 U per gram of yeast material (5h/pH 7.5/55ºC, 20% w/v yeast material suspension) was used (Magnani et al., 2009). For the inactivation of the enzyme at the end of this step, the temperature was raised at 85ºC for 15 min. The suspension was cooled down to 28ºC and centrifuged (5000 rpm/10 min).

2.2.2.6. Lyophilization
The lyophilization was performed as in 2.2.1.4.

2.2.2.7. β-Glucan production
With extraction Method 2, four different insoluble *β*-glucans were produced and named respectively g-5, g-6 g-7 and g-8 (Table 2).

2.2.2.8. Yield calculation of produced β-glucan
The yield calculation was performed as in 2.2.1.6

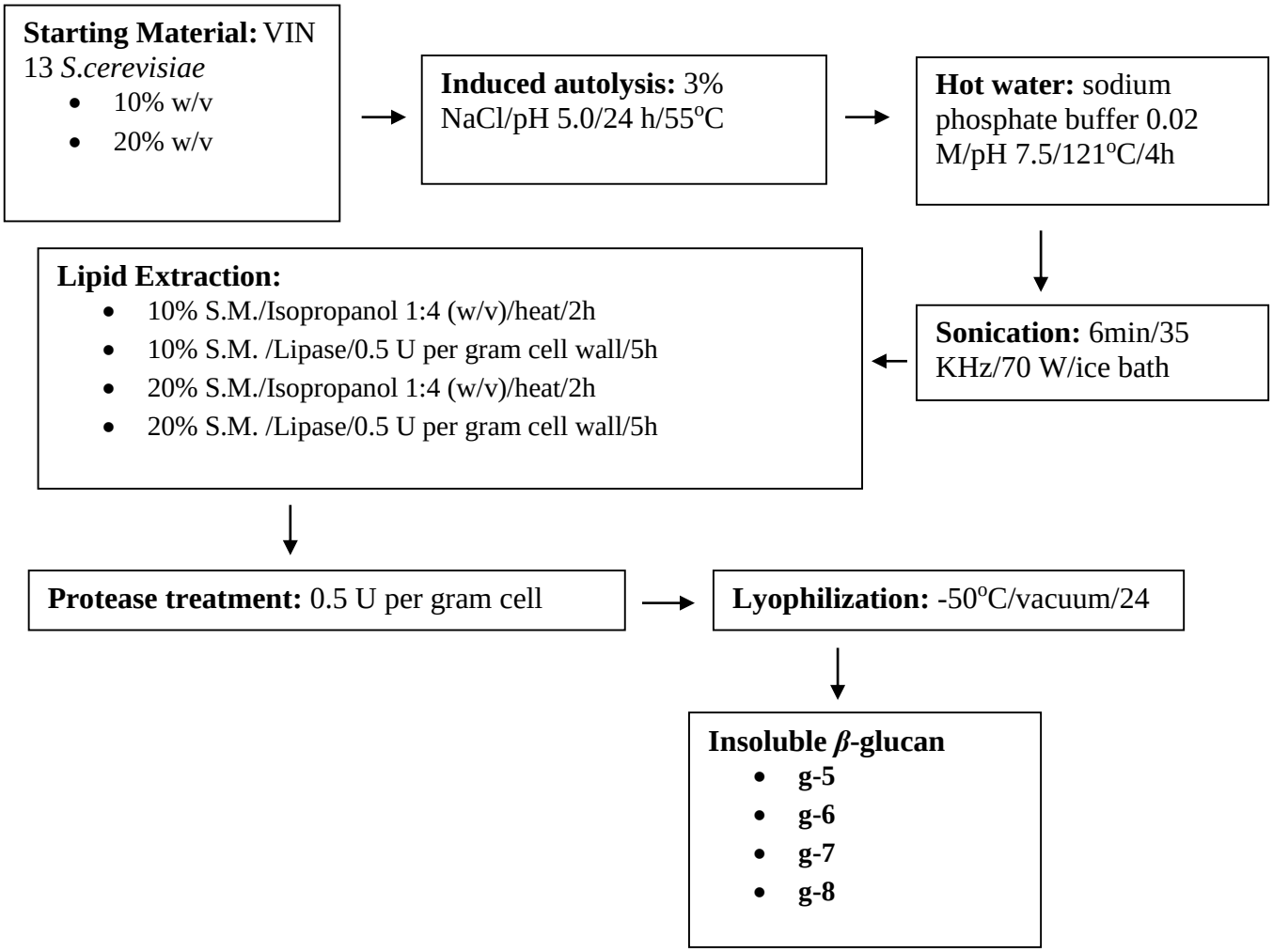

Fig. 2. Schematic diagram showing the process for the extraction of (1,3)(1,6)-*β*-D-glucan with extraction Method 2

2.2.3. Extraction of (1,3)(1,6)-β-D-glucan: Method 3

β-Glucan was isolated from yeast cell walls by a modification of the method of Zechner-Krpan et al., 2010 (Fig. 3).

2.2.3.1. Starting material-Yeast cells preparation/rehydration

20 g of dry yeast VIN 13 were divided in two equal portions (each of 10 g) and then diluted to dionized water leading to yeast cells slurry of 10% and 20% (w/v) respectively. This was done for the estimation of the appropriate rehydration of yeast cells which is affecting the extraction of the most extractable portion of *β*-glucan.

2.2.3.2. Induced autolysis

Induced autolysis was performed as in 2.2.2.1

2.2.3.3. Extraction and isolation of yeast β-glucan

The isolation of *β*-glucan from the yeast cell wall was done as previous (see 2.2.1.3.).

2.2.3.4. Lyophilization

The lyophilization was performed as in 2.2.1.4.

2.2.3.5. β-Glucan production
With extraction Method 3 four different insoluble *β*-glucans were produced and named respectively g-9, g-10 g-11 and g-12 (Table 3).

2.2.3.6. Yield calculation of produced β-glucan
The yield calculation was performed as in 2.2.1.6

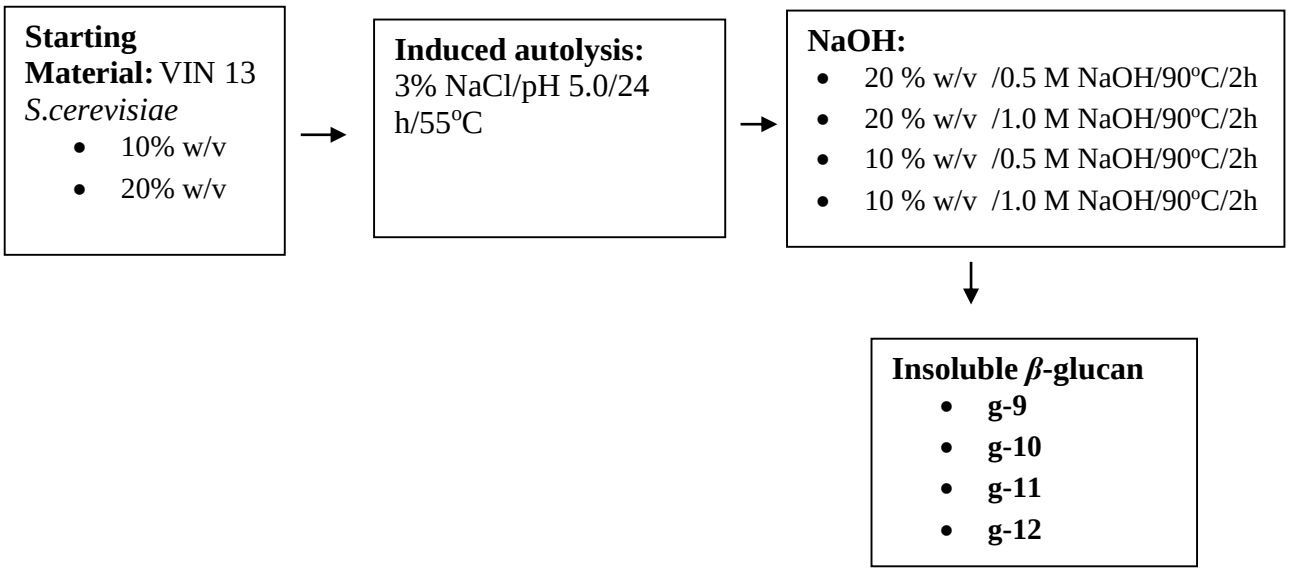

Fig. 3. Schematic diagram showing the process for the extraction of (1,3)(1,6)-*β*-D-glucan with extraction Method 3

2.2.4. Determination of β-glucan concentration
The determinations of total yeast *β*-glucan concentration in extracted yeast powder were performed with the use of Enzymatic K-EBHLG Yeast Beta-Glucan Assay Kit (Megazyme, Ireland)

2.2.5. Cell viability
Cell viability was determined using a haemocytometer (Neubauer type) using the methylene blue method (Logothetis et al., 2012). 1mL of sample medium was taken and diluted in 9 mL of deionised water. 1mL of this solution was dissolved with 1mL of 10% v/v methylene blue solution and left for 10 min. Aliquots of 1μL were placed on the haemocytometer by using a Pasteur pipette. The haemocytometer was then microscopically observed by an optical microscope (Olympus model CHK2-F-GS microscope). Yeast cell viability was calculated and expressed as follow:

$$\text{Viability (\%)} = a/n \times 100$$

where a: number of metabolically active cells
n: total cell number

3. Results and Discussion

3.1. Extraction of (1,3)(1,6)-β-D-glucan: Method 1
The first critical step during the process for yeast *β*-glucan extraction is the preparation of cell walls as *β*-glucans are located in that. For that purpose, cell lysis or disruption is necessary so that the cytoplasm flows out and cell walls are obtained. For the yeast

cell disruption many methods including mechanical, physical, chemical and enzymatic ways have been proposed. The second critical step is the cell wall purification which must lead to the less degraded part of glucose chains and distribution of *β*-glucan mass in supernatant and thus to reduced yield (Varelas et al., 2015). Glucanex® 200G contains mainly *β*-1,3-glucanase and some *β*-1,6-glucanase which are breaking down *β*-1,3-D-glucan and *β*-1,6-D-glucan biopolymer leading to the cell disruption. The resistance of yeast cell wall to lytic enzymes and the *β*-glucan content of yeast cell wall vary among the different species and the yeast growth conditions (Kwiatkowski et al., 2012; Kim et al., 2006; Aguilar-Uscanga et al., 2003). Three different concentrations of Glucanex® 200G were tested, 1, 10 and 50 time higher than the normal one (0.015 g/l) (Kim et al., 2006). The enzyme activity was tested by the measurement of cell viability at three phases, 0, 3 and 5 h after the enzyme application. Also, the pH of the supernatant at each of these phases was measured. After the lytic enzyme application, the mixture was centrifuged (5000 rpm/10 min), the supernatant was discarded and the sediment was used for the further step, the *β*-glucan isolation with hot NaOH.

After 5 h, the action of 10 times the enzyme normal concentration (E.N.C.) impacts negatively on the cell viability more than the application of one time the enzyme normal concentration (Fig. 4). At this time, 50 times the enzyme concentration caused almost the total disruption of yeast cells as the sediment received with centrifugation was found in traces and the total initial mixture was taken as a supernatant (Fig. 5). Also, the colour of each supernatant was different and a method based on spectrophotometry could be used for the estimation of the activity of the lytic enzyme and yeast cell lysis status (Fig. 5). The application of 50 times higher enzyme concentration than the normal one is affecting negatively the *β*-glucan production since the yeast cell is completely disrupted and the *β*-glucan is discarded in the supernatant. The recovery of *β*-glucan from the supernatant is difficult. It needs more purification steps as the supernatant is a mixture of *β*-glucans, mannoproteins, lipids, chitin and cytoplasm. The violence of yeast cell lysis is very important for the biotechnological production of *β*-glucans since there is an optimum where the cell is ‘mild’ disrupted and then can be further treated. The application and study of lytic enzymes can offer new perspectives in yeast cell wall resistance and the discovery of new antifungal drugs (Kim et al., 2006).
The pH of the supernatant 10 X E.N.C./5 h, was found 4.93 ± 0.02, a value near 5.0 were it is referred as the pH value where the yeast cell autolysis is successfully completed (de Palma Revillion et al., 2003; Yajun et al., 2003; Boonyeun et al., 2001) (Fig. 6).
The concentration of NaOH is affecting the final *β*-glucan concentration. The use of hot 0.5 M NaOH/2 h/90^{o}C leads to more pure fractions of *β*-glucan than the use of 1 M NaOH/2 h/90^{o}C. This NaOH concentration is in accordance with the reports of Javmen et al. (2012). The 0.5 M NaOH concentration differs from the reports of other researchers where 1 M NaOH concentration is proposed as more efficient (Zechner-Krpan et al., 2010; Suphantharika et al., 2003) but these researchers used different yeast strains and yeast material (e.g. brewer’s yeast waste). The reaction time of 2 h is shorter than 4 h reaction time that other researchers report (Javmen et al., 2012) but the used lytic enzyme was different. In our research, the 4 h NaOH extraction time affected negatively on *β*-glucan yield and purity and the values of these parameters were very low compared with the values of 2 h extraction time (data not shown). Also, the temperature 90^{o}C of hot NaOH differs from the value 100^{o}C that Javmen et al. (2012) propose, but these researchers used different material and lytic enzyme. Our temperature value is in accordance with the 90^{o}C value that other researchers propose as the most efficient (Zechner-Krpan et al., 2010; Suphantharika et al., 2003).

After hot NaOH, the mixture was centrifuged (5000 rpm/10 min) and the wet *β*-glucan (sentiment) was freeze-dried and weighted. The most purified fraction of *β*-glucan in the final yeast powder (44.18 ± 0.94 %) was achieved with the use 10% w/v dry yeast, yeast cell lysis with 10 X E.N.C./5 h and *β*-glucan isolation with 0.5 M NaOH/2 h (Fig. 7). Also, under these conditions the max. crude dry *β*-glucan (2.2 ± 0.06 g) and the max yield (9.71 ± 0.27 %) was obtained.

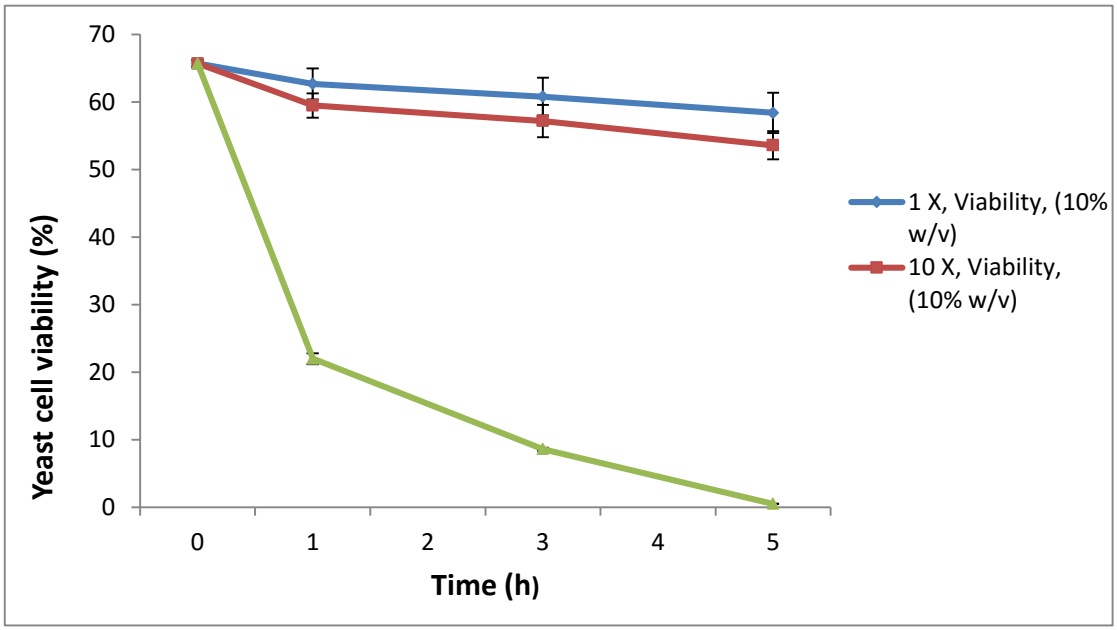

Fig. 4. VIN 13 yeast cell viability (%) after 0, 1, 3 and 5 h treatment with Glucanex® 200G, 1 time enzyme normal concentration, 10 times enzyme higher than the normal concentration and 50 times enzyme higher than the normal concentration (*n* = 3 replications for each viability and error bars represent standard errors of the average value of all replications with each range of viability).

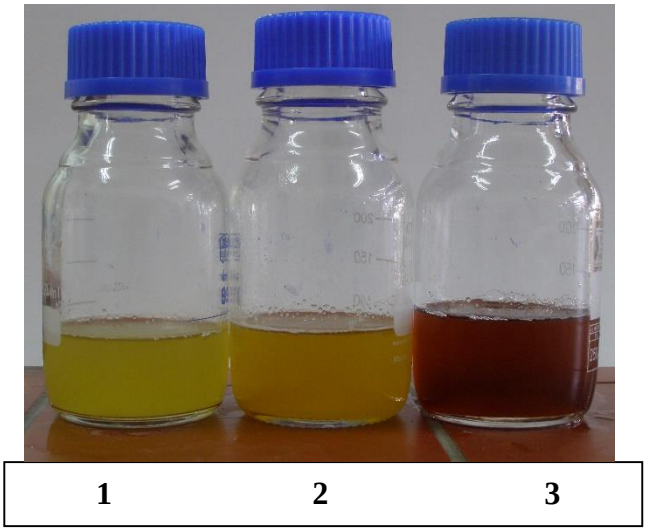

Fig. 5. Different volume and colour of supernatant after yeast cells treatment with Glucanex® 200G: 1) 1 time enzyme normal concentration; 2) 10 times enzyme higher than the normal concentration; 3) 50 times enzyme higher than the normal concentration

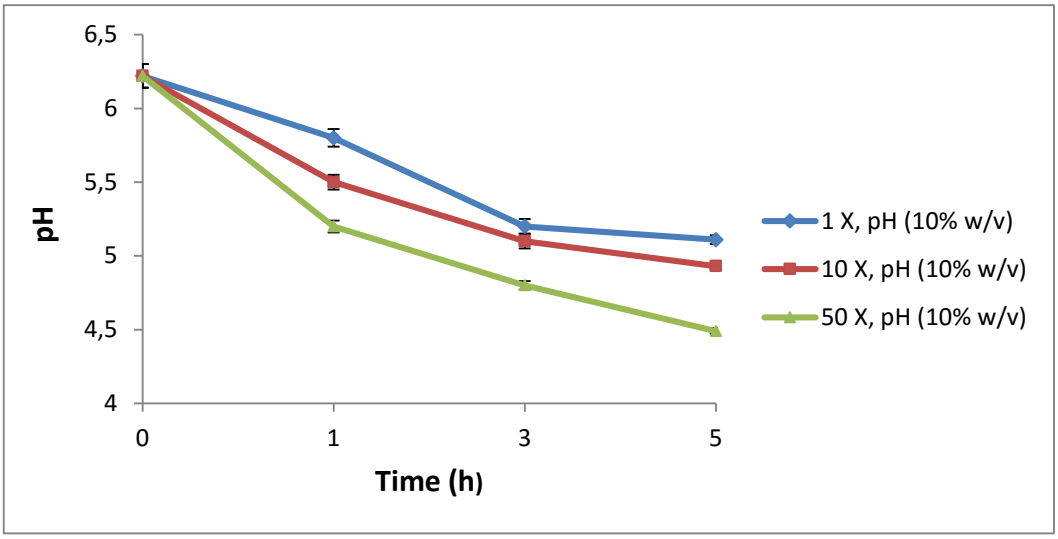

Fig. 6. pH of supernatant after 0, 1, 3 and 5 h treatment with Glucanex® 200G, 1 time enzyme normal concentration, 10 times enzyme higher than the normal concentration and 50 times enzyme higher than the normal concentration (*n* = 3 replications for each pH value and error bars represent standard errors of the average value of all replications with each range of pH value).

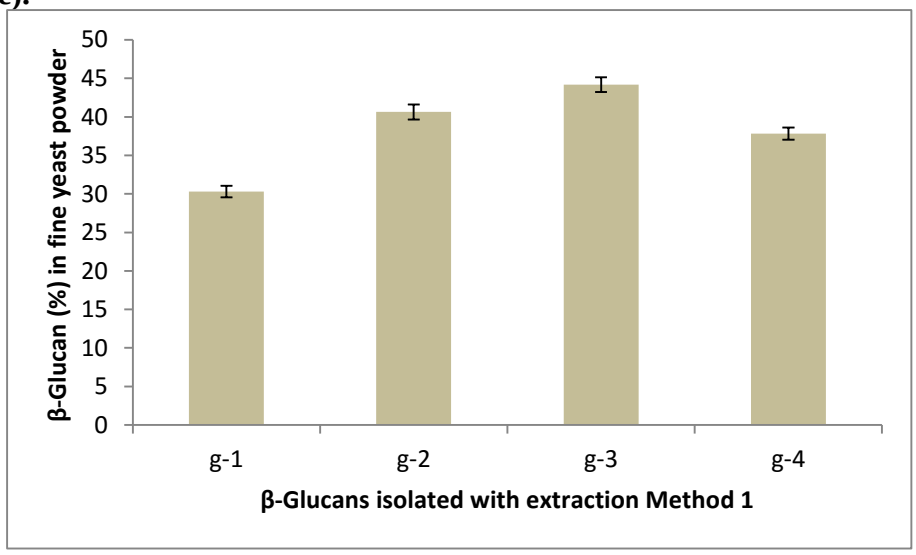

Fig. 7. VIN 13 yeast cells *β*-glucan concentration (%) after Extraction Method 1 process. The four different *β*-glucans produced during the extraction process are meant with g-1 to g-4 respectively (*n* = 3 replications for each concentration and error bars represent standard errors of the average value of all replications with each range of concentration).

β-glucan	Glucanex 200G	NaOH
g-1	1X	0.5 M
g-2	1X	1.0 M
g-3	10X	0.5 M
g-4	10X	1.0 M
-	50X	No further treatment
-	50X	No further treatment

Table 1. The critical steps which influence *β*-glucan production during extraction Method 1.

3.2. Extraction of (1,3)(1,6)-β-D-glucan: Method 2

In this method, the yeast cell lysis and the preparation of yeast cell walls was achieved with yeast autolysis (Yajun et al., 2003; Boonyeun et al., 2001). The autolysis procedure was tested against two different yeast cell suspensions, 10 and 20% w/v. Yeast autolysis was performed according to Magnani et al. 2009 and Liu et al., 2008 with 3% NaCl as an autolysis promoter, pH 5.0/55ºC/24 h. Yeast autolysis was estimated with the determination of R (%) autolysis ratio and the cell viability (%) (Liu et al., 2008).
The cell suspension plays an important role during autolysis. According to the manufacturer's instructions, the maximum cell rehydration is achieved with 10% (w/v). The cell rehydration is triggering the cell's enzyme systems properly via the water efflux through the lipid bilayer and finally is leading to a cellular reprogramming (Logothetis et al., 2013). The two different cell suspensions had almost the same effect on yeast viability at the end of a 24 h yeast autolysis process but the autolysis ratio R (%) was higher (29.21±1.5 %) for 10 % (w/v) cell suspension than 20 % (w/v) cell concentration (25.14±1.8 %). These values differ from other researcher's results but in their studies, the cell suspension was different (30 and 15% w/v) and the starting material was 28% on dry basis pressed mass of yeast and spent brewer's yeast slurry respectively (Magnani et al. 2009; Liu et al., 2008). Also, the differences on the impact of cell rehydration may arise from the fact that in our study, the starting material was dry yeast cells used for starting fermentation and in the other study the starting material was wet spent yeast cells after an alcoholic fermentation which are already hydrated (Liu et al., 2008). Finally, the cell suspension had a minor impact on the pH value of the supernatant of both cell suspensions at the end of the autolysis process. The pH was found near 5.5 for both cell suspensions and differs from the pH 5.0 reported from other researchers when the autolysis process is completed. In our study, the duration of the process was 24 h instead of 36 h in the other researchers' study where the R (%) is reported 42 % (Yajun et al., 2003). These differences are in accordance with our results showing that the duration of 24 h is short for the completion of the *S.cerevisiae* VIN 13 strain autolysis.

The removal of mannoproteins was performed according to Magnani et al., 2009 and Liu et al., 2008 with the treatment of yeast cell material with hot water. The cell viability test showed a slight decrease of viable cells after hot water treatment for both initial yeast cell suspensions (Fig. 8).
In the next step, the application of ultrasounds had a significant impact on cell viability with the decrease of the non-stained viable cell (Fig. 8).
In the next step, lipids were removed with two different ways, the use of isopropanol (Magnani et al., 2009; Liu et al., 2008) and the use of lipase Lipopan Extra (Novozymes, Denmark). This was done for the use of an alternative to the non-environmental friendly organic solvents which are not suitable for the production of food grade β-glucan and produce industrial waste when used in large quantities for β-glucan production. The use of lipase enzyme for the removal of lipids is affecting negatively to the final concentration of β-glucan in the final yeast powder. The use of isopropanol with 10 % (w/v) yeast cell rehydration gives the highest concentration of β-glucan in the final yeast powder (Fig. 9).
Finally, the proteins were removed with protease Protamex (Novozymes, Denmark)/0.5 U per gram of yeast material (Magnani et al., 2009).
In extraction Method 2, the 10% (w/v) cell suspension with isopropanol treatment gives the highest β-glucan concentration in the final yeast powder (42.39 ± 0.42 %) (Fig. 9). Also, under these conditions the max. yield (8.10 ± 0.42 %) was obtained. The max.

crude dry *β*-glucan (4.81 ± 0.08 g) was obtained with 10% (w/v) cell resuspension and lipase treatment.

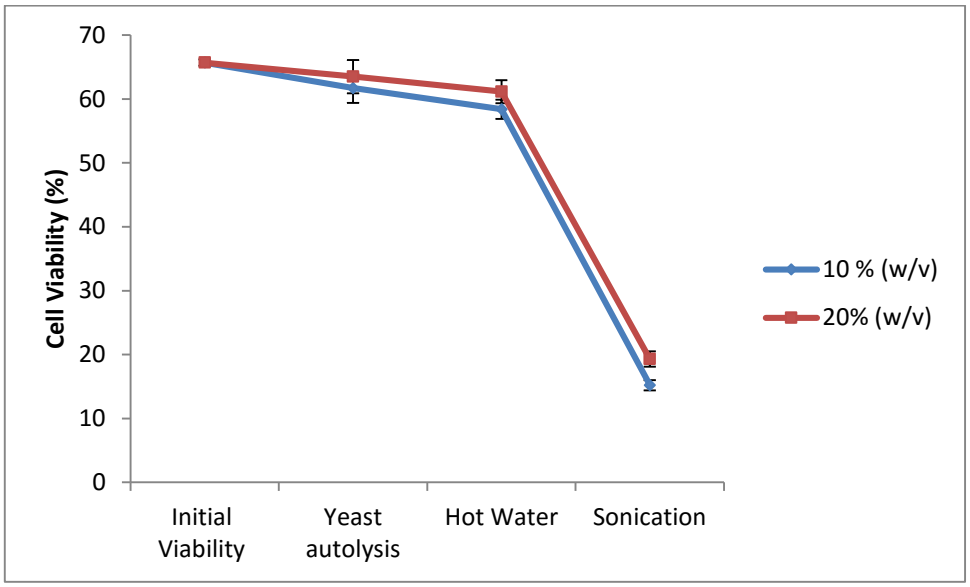

Fig. 8. Viability (%) of VIN 13 yeast cells during the phases of cell lysis and disruption (*n* = 3 replications for each viability value and error bars represent standard errors of the average value of all replications with each range of viability value).

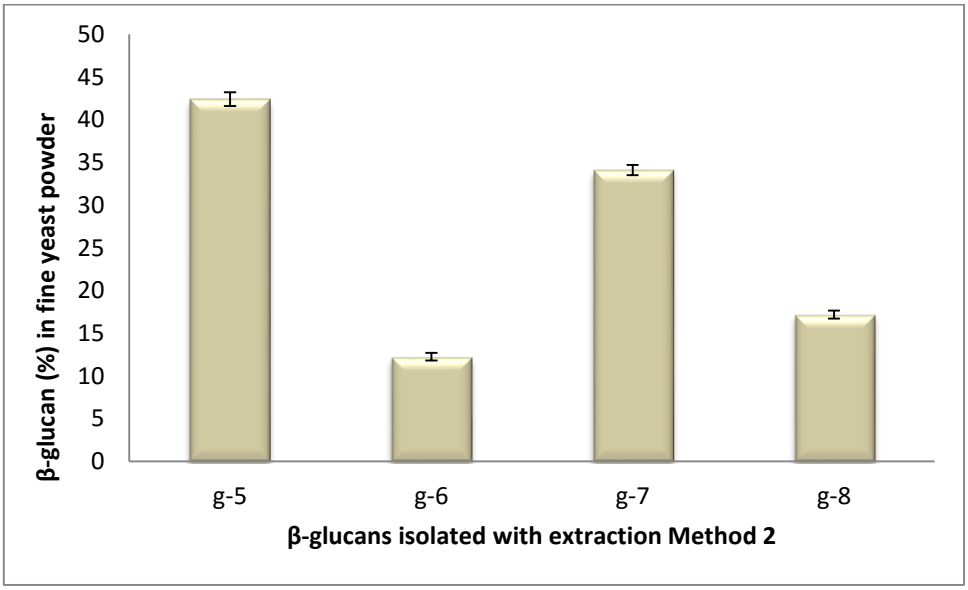

Fig. 9. VIN 13 yeast cells *β*-glucan concentration (%) after Extraction method 3 process. The four different *β*-glucans produced during the extraction process are meant with g-1 to g-4 respectively (*n* = 3 replications for each concentration and error bars represent standard errors of the average value of all replications with each range of concentration).

β-glucan	Induced autolysis	Lipid Extraction
g-5	10% (w/v)	isopropanol
g-6	10% (w/v)	Lipopan Extra
g-7	20% (w/v)	isopropanol
g-8	20% (w/v)	Lipopan Extra

Table 2. The critical steps which influence *β*-glucan production during extraction Method 2.

3.2. Extraction of (1,3)(1,6)-β-D-glucan: Method 3

The results of yeast autolysis have been analyzed in the previous section concerning extraction Method 2. The R (%) values (28.57% and 24.37% for 10% and 20% cell suspension respectively) were similar to the ones of Method 2. These results cannot be compared with the ones of Zechner-Krpan et al., 2010 as in their study the results of yeast autolysis are not referred.

The results of the effect of concentration of NaOH on the final *β*-glucan concentration show that the use of hot 0.5 M NaOH/2 h/90ºC leads to more pure fractions of *β*-glucan than the use of 1 M NaOH/2 h/90ºC. This differs from the reports of other researchers where 1 M NaOH concentration is proposed as more efficient (Zechner-Krpan et al., 2010; Suphantharika et al., 2003). Also, the value of the purity of the extracted *β*-glucan differs (64.56 % instead of higher than 90%) but these researchers used different yeast material (e.g. brewer's yeast waste) and different method for yeast *β*-glucan determination (Zechner-Krpan et al., 2010). In extraction Method 3, the 10% (w/v) cell suspension with 0.5 M NaOH gives the highest *β*-glucan concentration in the final yeast powder (64.56 ± 1.25 %) (Fig. 10). Also, under these conditions the max. yield (10.01 ± 0.42 %) was obtained. The max. crude dry *β*-glucan (6.65 ± 0.07 g) was obtained with 20% (w/v) cell resuspension and 0.5 M NaOH.

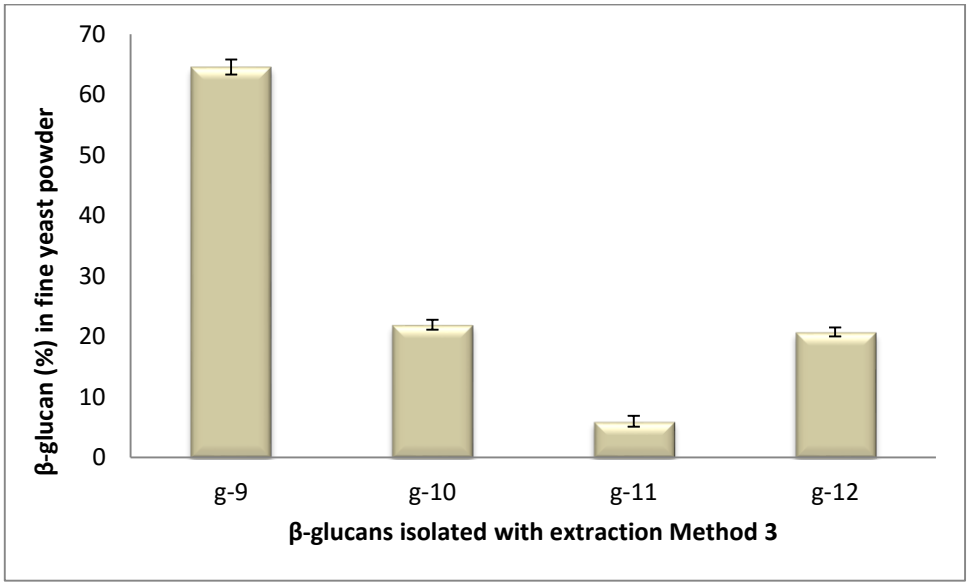

Fig. 10. VIN 13 yeast cells *β*-glucan concentration (%) after Extraction Method 3 process. The four different *β*-glucans produced during the extraction process are meant with g-9 to g-12 respectively (*n* = 3 replications for each concentration and error bars represent standard errors of the average value of all replications with each range of concentration).

***β*-glucan**	**Autolysis**	**NaOH**
g-9	10% (w/v)	0.5 M
g-10	10% (w/v)	1.0 M
g-11	20% (w/v)	0.5 M
g-12	20% (w/v)	1.0 M

Table 3. The critical steps which influence *β*-glucan production during extraction Method 3.

Conclusions

The max. cocncentration (64.56 ± 1.25 %) of *β*-glucan in the final yeast powder and the max. yield (10.01 ± 0.42 %) is achieved with the extraction Method 3 and more precisely with 10% (w/v) yeast cell rehydration, 24 h yeast autolysis and 0.5 M NaOH/2 h/90ºC. The mentioned method is easy, fast and low cost but it is more difficult and expensive to obtain more than 65% pure fractions. Further yeast powder manipulations and purifications can probably give *β*-glucan of even more higher concentrations since no effort for lipids and/or protein removal was tested.

This method seems to be suitable for the production of large quantities of crude *β*-glucan. We propose that this method could be used for the treatment of winery solid wastes (wine lees) for the production of *β*-glucan from yeast biomass remaining in the fermentation tanks. The proposed extraction method could comprise a pilot scale method for the optimization of a new method for *β*-glucan recovery from winery wastes.

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