

Researches concerning the preparation of spent brewer's yeast β -glucans

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Abstract

The work presents the preparation of spent brewer's yeast β -glucans by chemical and thermic treatment of yeast cell wall with NaOH solution at different temperatures. The yeast cell wall obtained by autolysis or ball milling of the brewer's yeast was used as raw material for the preparation of β -glucans. It has been demonstrated that a single alkaline extraction using 1,0 N NaOH solution at 90 °C leads to the highest efficiency. It carried out a β -glucan product with high carbohydrate content of 88,7 %, β -glucan content of 50,4 % (w/w) and with a low protein content of 2,9 % (w/w). The β -glucan content was determined by chemical assays.

Keywords: β -Glucan, brewer's yeast, alkaline extraction, cell wall

1. Introduction

Spent brewer's yeast biomass, a by-product from a brewery can be revaluated because it has a valuable chemical composition, available for human and animal feeds and health. The yeast cell wall has precious components such as the polysaccharides with many applications in the food industry and pharmaceutical domain [17,18,20,22].

In Romania, in the past, a large spent yeast amount was used as animal feed, but a lot of farms are closed today and the biomass is overflowed in the city sewage and pollutes the wastewaters. Therefore, the spent brewer's yeast revaluation is necessary and very important as an environmental protection measure, too.

The composition and structure of the yeast cell wall vary with species [14]. The cell wall of *Saccharomyces cerevisiae* is mainly composed of β -glucans (~50%) and mannoproteins (~40%). It also contains small amount of chitin and lipids. β -Glucan is a polymeric compound of glucose bonded via β -(1,3)- or β -(1,6)-D-glycosidic linkages [1,2,3,6,12,22].

β -Glucan plays an important role in the immune system and skin protection. It also has potent antioxidant properties and free radical scavenging capabilities. In addition, it increases the effectiveness of antibiotics and reduces the LDL cholesterol level in the body. β -Glucan has several chemotherapeutic effects which include the inhibition of tumor development, enhancement of defense against bacterial, viral, fungal and parasitic challenges and the activation of macrophages [4,8,9,10,21].

The classical preparation method of yeast β -glucans derived from *Saccharomyces cerevisiae* is a very complicated process consisting of several steps of alkaline, acid and organic extraction and several washing steps [7]. A less complicated procedure for preparation of spent brewer's yeast β -glucans was developed in this work.

2. Materials and Method

2.1. Brewer's yeast

The yeast used was a spent brewer's yeast slurry (a strain of *Saccharomyces uvarum*), a by-product from a brewery, with a solids content of ~ 20% provided by a Brewery from Romania.

The spent yeast was washed three times with three water volumes then it was treated with 0,25M Na₂CO₃ solution for reducing the bitterness. After washing by centrifugation the yeast biomass moisture was 18,43% for yeast Generation 4 (after four fermentation cycles).

2.2. Chemicals

Reagents and chemicals were obtained from Sigma Chemical Co., St.Louis, USA, Merck KGAA, Darmstadt, Germany and other biochemical company of Romania.

An enzymatic glucose kit was obtained from Megazyme International Ireland Ltd. and an amyloglucosidase enzyme from S.C. Enzymes & Derivates S.A. Costisa-Neamt, Romania.

2.3. Apparatus

Stereomicroscope Optika serie BM, Microscope Quanta 200 by Philips with Scanning Electron Microscopy, Mixer Mil Type MM 301 with stainless steel balls, Analytical balance Mettler Toledo xs 403 SM, Spectrophotometer UV/VIS-JASCO V530PC, pH-meter Mettler Toledo S20 K, Centrifuge JANETSKI with refrigeration, Shaking incubator SI 300 R, Water Bath, Kjeldahl analyzer, Thermobalance MX50.

2.4. Spent brewer's yeast cell wall preparation

Ball milling: the spent yeast with water (a 1:1 (w/v) ratio) was milled for 20 minutes (with intermittences) at 30 Hz frequency with 5 stainless steel balls with 12 mm diameter then the mixture was centrifuged for 15 minutes at 3300 rpm [6].

The solid residue is brewer's yeast cell wall obtained by milling (MCW). Autolysis: the spent yeast with water (a 1:1 (w/v) ratio) was maintained with continuous stirring for 24 hours to 50 °C at pH 5 then 24 hours to 60 °C at pH 3,5. The 1N HCl solution was used for pH adjustment. The autolysate sample were centrifuged for 15 minutes at 3300 rpm. The solid residue is brewer's yeast cell wall obtained by autolysis (ACW).

2.5. Spent brewer's yeast β-glucans preparation

50 g wet weight of the yeast cell wall ACW was suspended in various volumes of NaOH solution with different concentrations at various temperatures for different times periods with continuous stirring and then cooled to room temperature (Table 1) [19].

The mixture was centrifuged for 15 minutes at 3300 rpm and the supernatant was poured off. The solid residue was washed three times with distilled water (1:5 (w/v) ratio) and recovered by centrifugation after each washing. The solid residue is brewer's yeast β-glucans obtained by a single step alkaline extraction of spent brewer's yeast cell walls (AYG or MYG) [19].

Optimization of extraction conditions was carried out using four parameters (three parameters were fixed and one parameter was varied) for each treatment. In the first case was fixed: the NaOH concentration at 1N, the extraction time at 1h, the ACW to NaOH solution ratio at 1:5 and was varied the temperature at 50, 70 and 90°C (Figure 1). Identical algorithm is valid for other three cases (Figure 2,3,4) [19].

The solids yield was calculated and carbohydrate, protein, glycogen, β-glucans contents of the AYG or MYG were assayed after each treatment (Table 2).

The optimal alkaline extraction conditions of spent brewer's yeast cell wall obtained by autolysis or by milling were applied for several spent brewer's yeast cell wall samples for to achieve the decisive results in this study. It was made then a comparison between the results obtained for AYG and MYG (Table 2, Fig. 6).

2.6. Chemical assays

Solids content of samples was estimated by using a thermobalance for moisture analysis. Carbohydrate was determined after a hydrolysis procedure with 72% H₂SO₄ solution and 40 g/l Ba(OH)₂ saturated solution for acid solution neutralization [5,6] and reducing sugars were assayed with dinitrosalicylic acid (DNS) method. Total glucose was enzymatically determined with a glucose oxidase kit.

Glycogen was assayed enzymatically with amyloglucosidase and glucose oxidase kit. β-Glucan content was calculated by subtraction from the total glucose of the glucose obtained from glycogen [19]. Total nitrogen was determined by Kjeldahl method and protein content was calculated by multiplying the total nitrogen by 6,25.

3. Results and Discussions

In this study, an essential preoccupation was the manufacture of insoluble yeast cell wall material with high β-glucan content but low protein content by less complicated procedure. A single-step alkaline extraction of brewer's yeast cell wall achieved after

ball milling or autolysis was developed. Treatment with NaOH solution hydrolysed and solubilised the cellular proteins, nucleic acids, mannans, polar lipids and soluble β -glucans that pass into supernatant. The insoluble β -glucan is left in the solids fraction after centrifugation [19]. The alkali extraction conditions were optimized on a temperature, extraction time, alkali concentration and a cell wall to alkali ratio. In Fig. 1,2,3,4 is shown three chemical assays for cell wall carried out by autolysis alone (ACW). The results for MCW are indicated in Table 2.

When the NaOH concentration is fixed at 1N, the extraction time at 1h, the cell wall to NaOH solution ratio at 1:5, the extraction efficiency improved with increasing temperature as shown in Fig.1 and Tabel 2.

The highest β -glucan content (50,4%), carbohydrate content (88,7%) and lowest protein content (2,9%) were obtained at a temperature of 90 °C in case of AYG3 product.. A temperature of 50°C led to products with high protein content (0,3%) and high solids yield (36,2%) because the protein is not dissolved in supernatant at low temperature. The less good results are indicated in Table 2 concerning the MYG3 product because the cell wall disruption by ball milling is less effective comparative of the cell wall disruption by autolysis method.

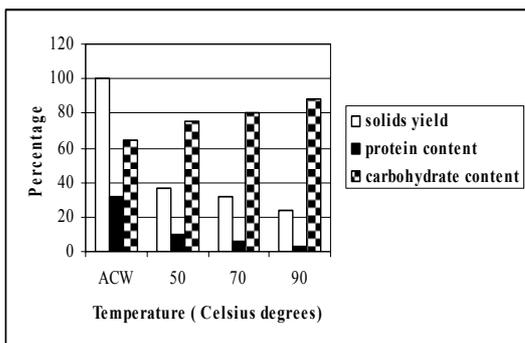


Figure 1. Effects of temperature upon solids yield, protein content and carbohydrate content at a sample to alkali solution ratio of 1:5, with 1,0 N NaOH solution, for 1 h extraction time.

Fig.2 shows that optimum extraction time as for carbohydrate content and solids yield was 1 hour. The carbohydrate content (88,7 %, 88,6% respectively) and β -glucan content (50,2%) for 1,5 and 2 h in case of AYG1 sample and the results obtained at 1 h were very handy so as to an extraction process displayed in 1,5 or 2 h becomes

not economical for industrial conditions. The protein content and solids yield were handy for 1,5 and 2 h, too.

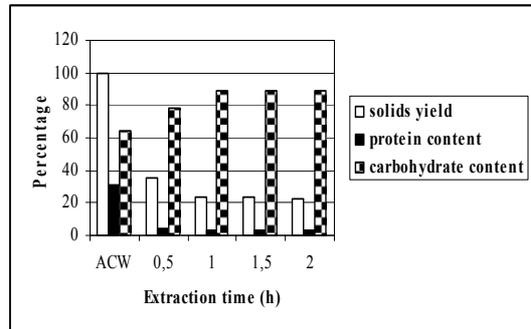


Figure 2. Effects of extraction time upon solids yield, protein content and carbohydrate content at 90 °C, with a sample to alkali solution ratio of 1:5 and 1,0 N NaOH solution.

The influence of the NaOH concentration on the carbohydrate and protein contents and solids yield of AYG is shown in Fig. 3. In this case it is noticed that the carbohydrate content has the biggest augmentation when the alkali concentration was increased from 0,5 to 1,0 N and then is too small the difference. The maxim account is 88,7% carbohydrate content and 50,4% β -glucan content for 1 N NaOH concentration and the minimal account is 2,9% protein content for same NaOH concentration. The solids yield was decreased to 24,1% based on dry weight of yeast cell wall.

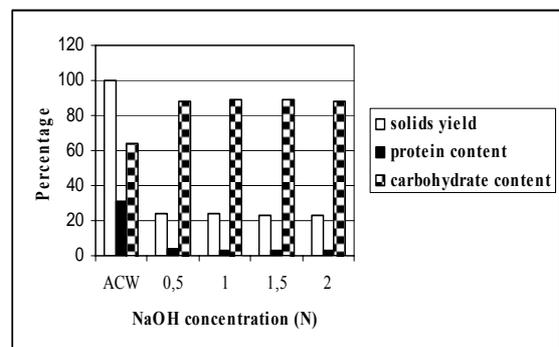


Figure 3. Effects of NaOH concentration upon solids yield, protein content and carbohydrate content at 90 °C, with a sample to alkali solution ratio of 1:5, for 1 h extraction time.

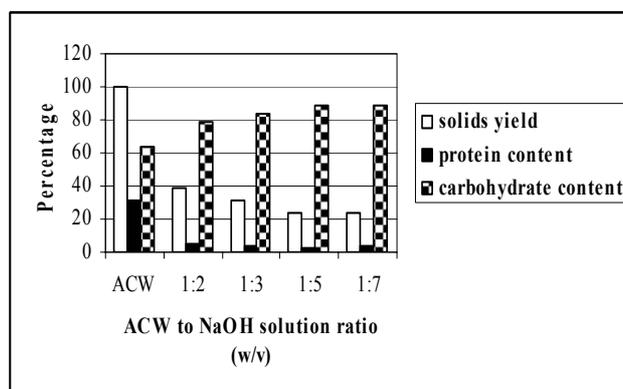
The important role of sample to alkali solution ratio 1:5 is perceived in Fig. 4 where the maxim value for carbohydrate content is 88,7% at 1:5 ratio (w/v) and the minimal value for protein content is 2,9% at same ratio. Such as in case of the extraction time, a sample to NaOH solution ratio 1:7 becomes not economical for an industrial process.

Table 1. The alkaline extraction conditions of spent brewer' yeast cell wall samples

Treated samples		Temperature (°C)	Time (h)	NaOH Concentration (N)	Sample/NaOH solution ratio (w/v)	Obtained samples	
ACW1	MCW1	90	2,0	1,0	1:5	AYG1	MYG1
ACW2	MCW2	90	1,5	1,0	1:5	AYG2	MYG2
ACW3	MCW3	90	1,0	1,0	1:5	AYG3	MYG3
ACW4	MCW4	90	0,5	1,0	1:5	AYG4	MYG4
ACW5	MCW5	50	1,0	1,0	1:5	AYG5	MYG5
ACW6	MCW6	70	1,0	1,0	1:5	AYG6	MYG6
ACW7	MCW7	90	1,0	1,0	1:2	AYG7	MYG7
ACW8	MCW8	90	1,0	1,0	1:3	AYG8	MYG8
ACW9	MCW9	90	1,0	1,0	1:7	AYG9	MYG9
ACW10	MCW10	90	1,0	0,5	1:5	AYG10	MYG10
ACW11	MCW11	90	1,0	1,5	1:5	AYG11	MYG11
ACW12	MCW12	90	1,0	2,0	1:5	AYG12	MYG12

Table 2. Chemical composition of yeast products

Sample		Solids yield %		Protein (TN x 6,25) % w/w dry basis		Carbohydrate % w/w dry basis		Glycogen % w/w dry basis		β-Glucan % w/w dry basis	
BYC		-		45,3		52,6		5,8		15,9	
ACW		100		31,4		64,2		10,3		22,5	
MCW		100		36,7		56,3		8,1		18,8	
AYG1	MYG1	22,5	40,0	3,1	12,4	88,6	77,2	29,6	32,7	50,2	37,5
AYG2	MYG2	23,4	41,2	3,2	12,6	88,7	77,2	30,1	33,1	50,2	37,9
AYG3	MYG3	24,1	42,5	2,9	11,8	88,7	77,4	30,4	34,7	50,4	38,1
AYG4	MYG4	35,4	53,1	4,3	16,4	78,1	65,9	20,5	25,6	40,6	31,1
AYG5	MYG5	36,2	53,4	10,3	28,3	75,4	64,8	18,3	22,5	37,2	29,5
AYG6	MYG6	31,5	47,3	5,6	18,5	80,6	69,0	24,4	25,7	43,2	34,2
AYG7	MYG7	38,6	55,2	4,4	16,7	79,3	67,7	24,3	24,1	44,1	32,5
AYG8	MYG8	30,7	47,0	3,7	13,2	83,4	72,1	27,6	29,5	46,3	36,7
AYG9	MYG9	24,0	41,7	3,3	12,8	88,4	76,8	29,1	32,6	48,9	36,9
AYG10	MYG10	23,8	40,3	4,2	15,7	88	76,7	28,7	32,5	49,0	37,2
AYG11	MYG11	23,5	40,1	3,1	12,3	88,6	77,1	30,0	33,8	49,7	37,8
AYG12	MYG12	23,2	40,5	3,0	12,2	88,4	77,0	29,5	34,1	50,1	38,0

**Figure 4.** Effects of sample to alkali solution ratio upon solids yield, protein content and carbohydrate content at 90 °C, with 1,0 N NaOH solution for 1 h extraction time.

This part of experiment advance the optimum conditions for a single-step alkaline extraction of spent brewer's yeast cell wall acquired after autolysis or ball milling : NaOH concentration - 1,0 N; extraction time - 1 h; temperature - 90⁰C; sample/ alkaline solution ratio – 1:5. These values for brewer's yeast *Saccharomyces uvarum* were in good agreement with the values found by Suphantharika et al(2003).

Compositional characteristics of various yeast products from spent brewer's yeast are summarized in Table 2. The assays is performed in triplicate.

First analyzed sample was the spent brewer's yeast whole cell that has the littlest (52,6%) carbohydrate, (5,8%) glycogen and (15,9%) β -glucan contents and the keenest protein content (45,3%).

A few chemical constituents of yeast cell were released after cell wall breakage by autolysis and ball milling so as to the ACW and MCW yeast derived products presents the bigger percentage for β -glucan content (22,5% and 18,8%) because the same β -glucan amount from whole yeast cell remain into cell wall and is reported to the smaller total compounds amount, logical. At the same time, the protein content decreases to 31,4% (ACW) and 36,7% (MCW) because the certain protean compounds were eliminated by autolysis or milling.

Finally, the last product (the desirable product) AYG or MYG was attained the highest β -glucan content (50,4% or 38,1%) whereas protein content was the lowest (2,9% or 11,8%) because a big percentage of mannoproteins and chitin was solubilised in NaOH alkaline solution.

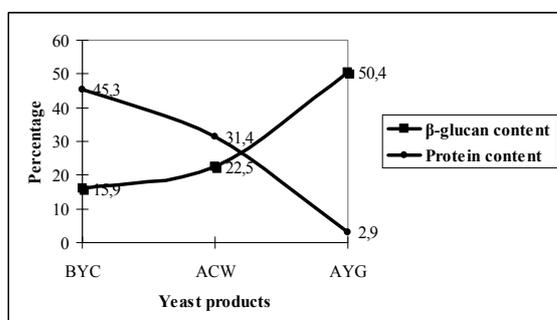


Figure 5. Evolution of β -glucan and protein contents in the spent brewer's yeast β -glucan preparation process

Fig. 5 shows the development of β -glucan content and the decreasing of protein content in the brewer's yeast products that represents the preparation processes steps.

It was made a comparative study between the production yields of β -glucan products from cell wall prepared by autolysis and milling, respectively and the graphical results are indicated in figure 6 that shows a bigger efficiency in the AYG case.

During of chemical analysis it was carried out concomitantly, a microscopic examination of the yeast derived products by scanning electron microscopy for to observe the cell wall disruption and the β -glucan product aspect at different magnifications and the microscopical pictures are reproduced in figure 7, 8, 9, 10. In fig.7 it is observed many yeast cell with broken cell wall and the intracellular content is in environs overflowed.

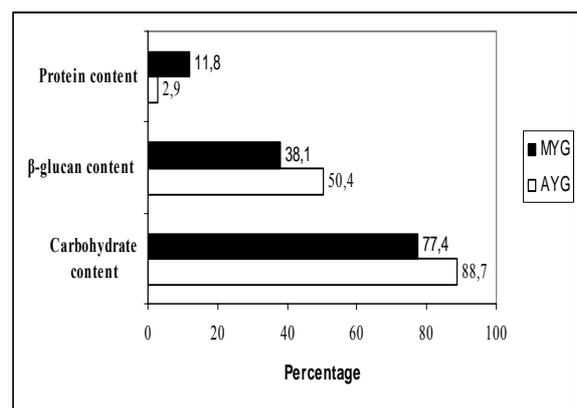


Figure 6. Chemical composition of β -glucan products AYG and MYG obtained in the optimal alkaline extraction conditions

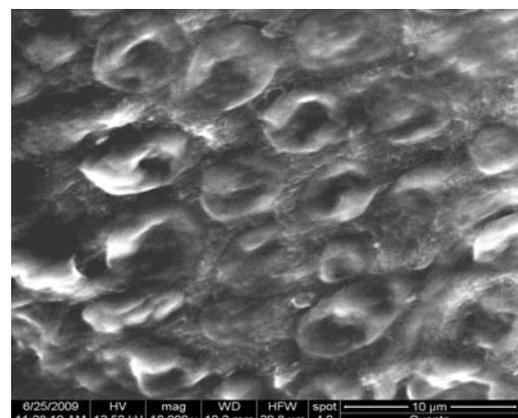


Figure 7. Group of disrupt and whole cells after ball milling at 10000 x magnification (MCW)

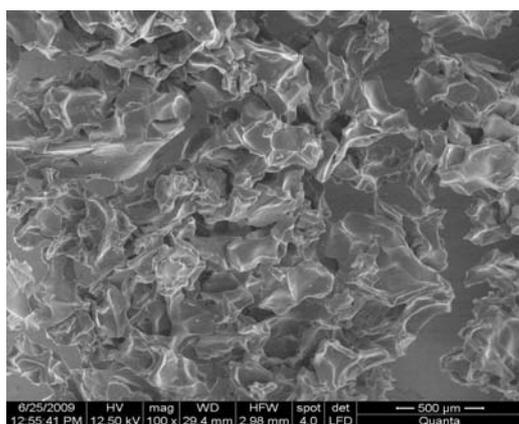


Figure 8. Spent brewer's yeast β -glucan at 100 x magnification

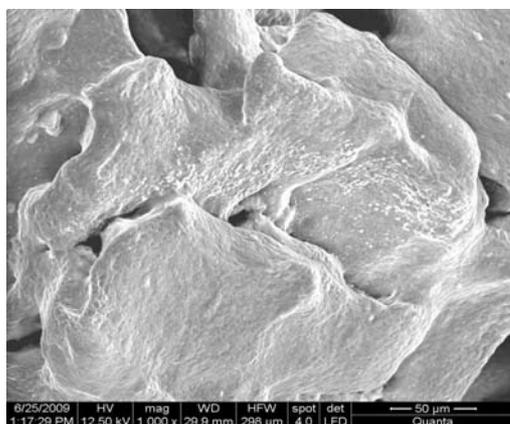


Figure 9. Spent brewer's yeast β -glucan at 1000 x magnification

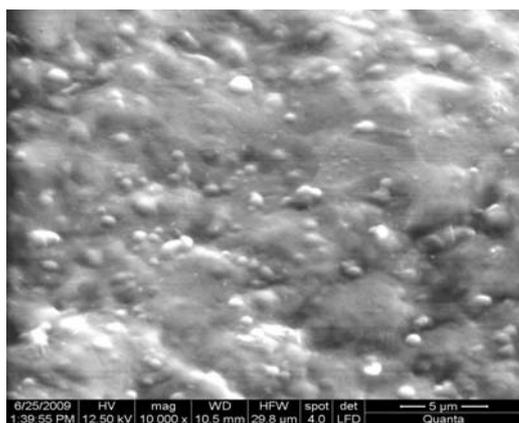


Figure 10. Spent brewer's yeast β -glucan at 10000 x magnification

4. Conclusions

The spent brewer's yeast can be revaluated by an extraction process of β -glucan, the polysaccharide with very important role in farmaceutical domain and food industrie.

An yeast derived product with $\sim 50\%$ (w/w) β -glucan content and $\sim 3\%$ protein content was obtained by chemical treatment with NaOH solution from spent brewer's yeast cell wall achieved by autolysis process.

This product was prepared by a single step alkaline extraction in the following optimum conditions: using 1,0 N NaOH at a temperature of $90\text{ }^{\circ}\text{C}$ for 1 h with a sample to alkaline solution ratio 1:5 (w/v).

For to achieve β -glucan yeast product can be used cell wall obtained by ball milling but with lowest efficiency (38,1% β -glucan content and 11,8% protein content).

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