

Acidic extraction of oat beta glucan by high purity

Abstract

Beta Glucan is considered to be a functional and bioactive substance, which can be added to many types of food products or taken as a food supplement in the form of capsules. In our work, we try to extract Beta Glucan from oat bran especially of the high concentration in the extract. We used acidic method to extract Beta Glucan and got final extract by concentration and received Beta Glucan content of 90%. So, now using a simple method you only need accuracy in work and you can get Beta Glucan of high concentration and use it in many food products.

Keywords: oat bran, aidic extraction, beta glucan extract, high concentration, bioactive material

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Introduction

Beta Glucan is a natural polysaccharide, which has soluble and insoluble types. The soluble type is of great importance.¹ So, we will discuss the extracting of the soluble Beta-D-Glucan, which is present in many cereal grains, yeast, fungus and some bacteria. It has many structural forms as (1→3)(1→4)-β-D-Glucan and (1→3)(1→6)β-D-Glucan, the first one is present in cereal grain and the second one - in yeast, fungus and some microorganisms.²

The importance of beta Glucan is that it can be considered as a functional and bioactive ingredient; it also has high preventive action against many diseases as atherosclerosis, diabetes militias, and cancer; it improves digestion and the health of normal microflora.³ For this now it is recommended to take not less than 3g daily to be able to act as bioactive ingredient.⁴ On the other hand, we can add it to many products to improve their quality and if they contain starter culture it improves its growth as in yoghurt, ice cream, bread, beer.^{2,3} In our research, we will extract beta Glucan of high purity from cereal (for example, oat bran).

Purpose

To extract beta-Glucan from oat bran of high purity percentage reaching 90% and to show the main difference between our method and other acidic extraction methods. It allows us to add the extract to different types of products and not to lose its functionality and nutraceutical effect. Also it is better to add the beta-Glucan itself than oat because it permits to add the beta-Glucan by low amount and achieve the daily level of human needs than to add the oat by very large amount.

Materials and methods

Materials (Table I)

Methodology

We will have 2 samples, each 20g of oat bran.⁵

- Weigh oat bran (20g).
- Prepare ethanol (80%), add by the ratio 1:4 (oat:ethanol).
- Mix them together and leave them on the magnetic stirrer for

2hours at room temperature (22-25°C) at 600rpm. pH=6,7 at 25°C.

- Then add NaOH (1M=4%) by ratio 1:7 (oat:NaOH).
- Mix them on the magnetic stirrer at 45°C for 2hours at 250rpm (Figure 1).
pH=12,8 at 21°C, nonhydrine test is positive, starch test is negative.

Nonhydrine test: Take an amount from the tested solution and add the same amount from the nonhydrine solution 0, 5% and heat for 2-3minutes. If it gives blue color, the result is positive (Figure 2).

Starch test: Take 2ml of tested solution and add 3drops of Lugol's iodine. If violet to blue color appears so than it is positive result (Figure 3).

Cool till 20°C then centrifugate at 6000rpm at 20°C for 15min and take supernatant (we see separation of 3layers of sediments, Figure 4).

Recentrifugate again at 6000rpm for 15min (we find little precipitation at the bottom). pH=12,8 at 27°C, nonhydrine test is negative.

Then add citric acid 15% to supernatant till pH reaches 3,5 at 20°C (it takes citric acid by ratio 1:1,13 citric acid to filtrate (Figure 5).

Cool till 4°C then centrifugate at 15000rpm for 30min at 4°C and separate the supernatant (Figure 6). pH=3,6 at 16,8°C.

Then add 80% ethanol by ratio 1:2 supernatant : ethanol (it is better to divide each 100 ml of supernatant and add ethanol because it increases yield and purity of beta Glucan). pH=4,5 at 25,2°C (Figure 7).

Leave it at 4°C for 15min then centrifugate at 6000rpm for 15min at 4°C then separate the sediment pellet (Figure 8). Put it into a Petri dish knowing its weight, and then weigh the extract.

Dry the pellets in a hot air oven at 42°C till they are completely dry and the color is slightly dark and not sticky (Figure 9). It takes from about 15 to 16hours. Then keep it in a clean dry small glass bottle. For better drying put on Petri dish and put it into the dryer as fast as possible because slow drying leads to the crystallization of Beta-Glucan and results in absorbing water. Then weigh the extract again.

Grind well till you get a powder (Figure 10), this also improves its solubility in water and usage by microflora. Then weigh it.

Then we prepare the sample for spectrophotometer analysis:^{6,7}

- i. Dissolve the extract in water at the temperature of 25-30°C at 700rpm by magnetic stirrer. It takes approximately 2hours to dissolve by a concentration of $5 \cdot 10^{-5}$ g/ml.
- ii. Prepare the reagent (cold 86% sulfuric acid, every 1 ml contains 0,7mg L-cysteine, Figure 11).

Remark: add the cysteine when the sulfuric acid is cold and leave on the stirrer at 250rpm for 30min to dissolve well in sulfuric acid. Do not leave them for a long time especially at warm temperature because

they react together and give yellow color and give false result.

- iii. Then add 2ml of reagent to each 400µl of dissolved extraction.
- iv. Put them directly into boiling water for 3min.
- v. Leave at a room temperature to cool for 40min before reading on spectrophotometer at 415nm and record the absorbance (Figure 12).
- vi. Compare the absorbance with the standard curve of glucose to identify the glucose concentration in our extract, and with the knowledge of the molecular weight of beta Glucan and glucose, we can determine the percentage of beta-Glucan in the extract.

Table I Materials

Bran oat	Ethanol 80 %	NaOH 1M (4%)	Citric acid (15%)	Sulfuric acid (86 %)	L-cysteine, petri dish
Distilled water	Centrifugator and its tubes	Magnetic stirrer, beds	Balance, cups, spoon	Flasks, test tubes	Grinder
Nonhydrine	Hot air oven	Lugol's iodine	Small glass bottle		



Figure 1 The mixture of oat bran, ethanol and NaOH on stirrer.

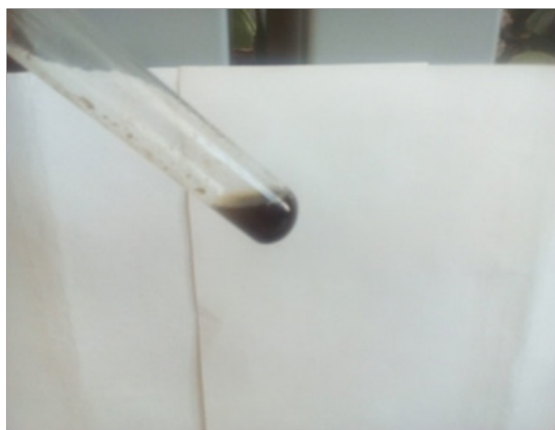


Figure 2 Nonhydrine test: positive result.

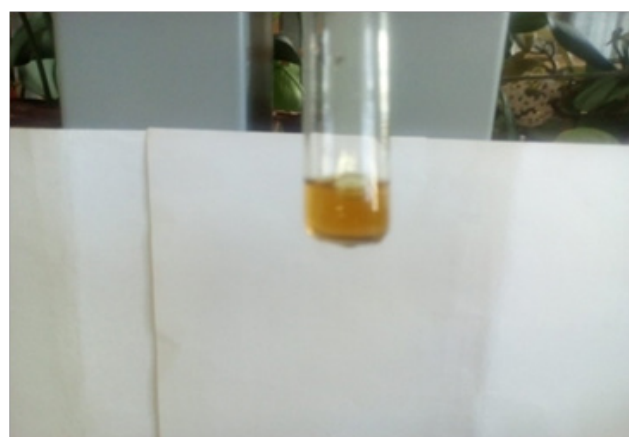


Figure 3 Starch test: negative result.



Figure 4 After first centrifugation at 6000rpm for 15min.



Figure 5 After adding citric acid and the pH becoming 3,5.



Figure 6 After centrifugation at 15000rpm for 30min.

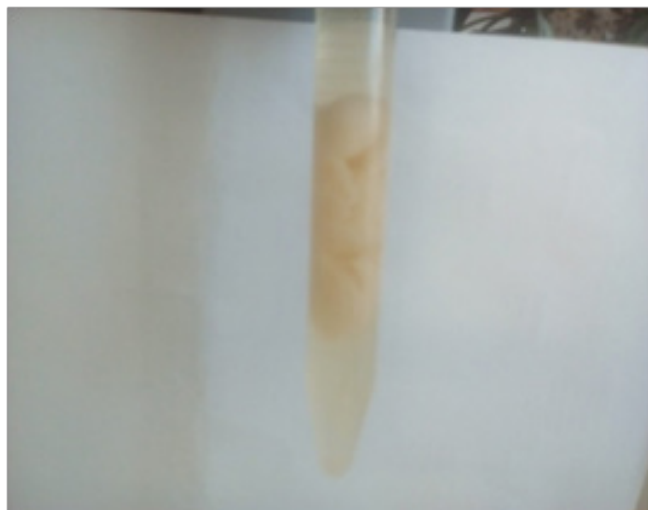


Figure 7 Beta-glucan after adding ethanol.



Figure 8 Beta-glucan after separation as pellet



Figure 9 Beta-glucan extract after drying.



Figure 10 Beta-glucan after grinding.



Figure 11 The reagent (sulfuric acid+L-cysteine) and dissolved beta-glucan.

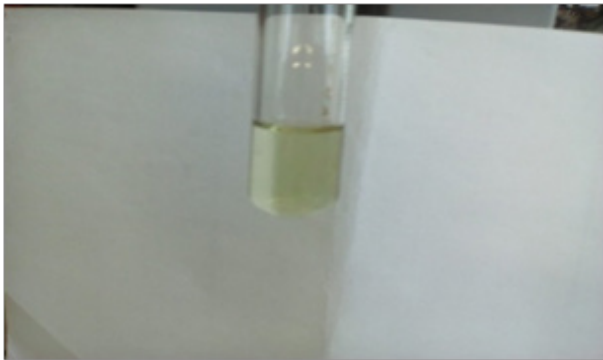


Figure 12 The tested solution after the 40min period.

Results and discussion

First sample

The weight of the Petri dish=121,590g

The weight of the Petri dish+Beta-Glucan before drying=124,144g

The weight of the Petri dish+Beta-Glucan after drying=122,224g

So the weight of our Beta-Glucan extract=0,634g

After grinding=0,628g

Second sample

The weight of the Petri dish=109,383g

The weight of the Petri dish+Beta-Glucan before drying=112,528g

The weight of the Petri dish+Beta-Glucan after drying=110,369 g

So the weight of extracted Beta Glucan=0,986g

After grinding=0.885g

Spectrophotometer analysis

We plot the standard curve (Figure 13 & Figure 14, curve 1) for glucose crystal (1mole glucose+1mole water) at concentrations ranging from $1,7 \cdot 10^{-4}$ to $0,5 \cdot 10^{-5}$ mol/l and using 415nm for absorbance measurement.

After, we use the same wavelength for the measurement of sample's solutions. Glucose concentration of the samples was derived from comparison of the absorbance values obtained from samples and the standard curve.

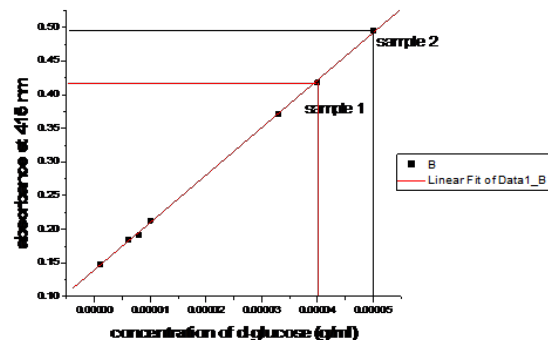
So, if the first sample has y (absorbance)=0.418 and the second sample has y (absorbance)=0.495.

So, from the equation, $y=6994X+0.140$.

The first sample has, X (glucose conc.)= $4 \cdot 10^{-5}$ g/ml and the second sample has, X (glucose conc.)= $5 \cdot 10^{-5}$ g/ml.

Calculation of the obtained Beta-Glucan concentration: If we know that the molecular weight of oat Beta-Glucan= $2 \cdot 10^6$ g/mol and the molecular weight of glucose=180g/mol.

In the first sample: glucose concentration= $4 \cdot 10^{-5}$ g/ml, so the same for Beta-Glucan in the same 1ml.



$$\text{Equation: } Y = A + B \cdot X$$

Parameter	Value	Error
A	0.1397	0.00212
B	7041.3244	76.32513

R	SD	N	P
0.99971	0.00363	7	<0.0001

Figure 13 Standard curve I (By system origin 61).

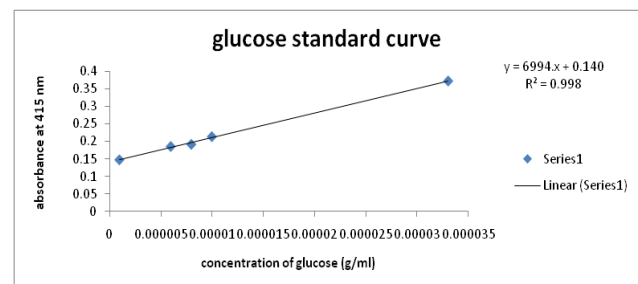


Figure 14 Standard curve I (By system Microsoft Office Excel 2007).

Because our glucose sample is crystal (180g glucose+18g water), so the concentration of pure glucose in it= $4 \cdot 10^{-5} \cdot 91/100=3,6 \cdot 10^{-5}$ g pure glucose. So the same of Beta-Glucan in the 1ml. And because we took $5 \cdot 10^{-5}$ g of our extraction, so this weight has the $3,6 \cdot 10^{-5}$ g of Beta-Glucan. So the purity of Beta-Glucan in first extraction was=72%

In second sample: Glucose concentration= $5 \cdot 10^{-5}$ g/ml, so the same for Beta-Glucan in the same 1ml.

Because the glucose sample is crystal (180g glucose+18g water), so the concentration of pure glucose in it= $5 \cdot 10^{-5} \cdot 91/100=4,5 \cdot 10^{-5}$ g pure glucose. So the amount of Beta-Glucan is the same in 1ml. And because we take $5 \cdot 10^{-5}$ g of obtained extraction, so this weight has the $4,5 \cdot 10^{-5}$ g of Beta-Glucan. So the purity of Beta-Glucan in the second extraction was=90%

Finally, we can say that this difference comes from decreased losses and decreased time between steps, also the supernatant received after the adding acid must not be left for a long time before adding ethanol to it and try to centrifugate quickly. Also the increase of the weight of the extract itself between the two samples is clear. On the other hand, it is known that the oat bran contains nearly 6% of Beta-Glucan, and we take 20g each time so it contains 1,2g Beta-Glucan.

In the first sample, we extract 0,634g of 72% purity, so we extract 0.5g of Beta-Glucan which is in the oat bran. In the second sample, we extract 0,986g of 90% purity, so we extract 0.9g of Beta-Glucan which is in the used oat bran. We see that the amount of Beta-Glucan we succeed to extract in the second sample is high and we decrease the loss of Beta-Glucan.

Conclusion

Thus, in the present work we successfully carried out the extraction of Beta-Glucan from oat bran. And showed that this extraction can be very high in the purity and by good amount of extract, but it needs a lot of care during extraction. Nowadays, we can find it also as capsules in pharmacy as a preventative means of diseases and to improve human's normal microflora.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

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