COMPARISON OF THE RESPONSIBILITY TO EXTRACT AND PURIFY β - GLUCAN OF SOME CEREAL GRAINS FOR SPECIFIC METHOD

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ABSTRACT

The current study prioritized certain popular local cereal species like Wheat, Oat, Rye and two local Barley varieties known as Aksad and Amal to extract their β- glucan using alchemical methods. The results of this study indicated that rye contained the lower percentage of β-glucans (0.756%) whereas, both types of barley Aksad and Amal contained highest amount (9.489%) and (7.395%) respectively. However, the results showed that the crude extracted of β- glucans from wheat, oat, rye, barley (Aksad) and barley (Amal) were 0.373,1.409, 0.26, 2.287 and 1.855 g/100g flour respectively which was reflected the efficiency of extraction process which ranged between 8.83 ± 0.01for wheat to 34.39 ± 0.02 for rye, the precipitating by ammonium sulfate were useful for purifying of oat and barley β- glucan which their purity increased to about 10- 15% to reach their purity to 84.88 for barley (Amal) to 95.18% for oat. All the bands of Ft-IR Spectra showed to take place absorbance at these regions by β-glucan are present in the all scanned studied samples with some differences in their areas and absorbance. A robust and sharp peak at 1304.37 cm^{-1} appeared only with the control sample while it is absent in the studied β-glucan. Results of HPLC illustrated that all purified cereal β- glucan appeared only one peak at the same Retention time RT of standard oat β- glucan which ranged between 17.429 to 17.453 min.

Key words: β-glucan; extraction; purification; quantification of β-glucan, Ft-IR Spectra, detection of β-glucan.
Part of PhD Dissertation of the 1st author.

مقارنة لقابلية الاستجابة لاستخلاص وتنقية بيتا كلوكان من بعض حبوب المحاصيل بطريقة محددة

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المستخلص

شرعت هذه الدراسة باستخدام بعض أصناف حبوب المحاصيل المحلية الأكثر استهلاكا شعبيا مثل الحنطة والشوفان والشيلم وصنفين من الشعير هما اكساد وامل لاستخلاص البيتا كلوكان منها باستخدام الطرق الكيمائية. أظهرت نتائج هذا البحث ان محتوى البيتا كلوكان في الشيلم كان الأقل (0.756٪) بينما كان الاعلى في صنفي اكساد (9.489٪) وامل (7.395٪). واظهرت النتائج أيضا ان المستخلص الخام من البيتا كلوكان كان للحنطة (0.373) وللشوفان (1.409) والشيلم (0.26) ولصنفي الشعير اكساد (2.287) وامل (1.855) غم \100غم طحين، وهذه القيم عكست كفاءة الاستخلاص التي كانت بين 0.01±8.83 للحنطة و0.02 ± 34.39٪ للشيلم، كما ان الترسيب بكبريتات الامونيوم زاد من كفاءة التنقية بمقدار15– 10٪ لتصل النقاوة الى84.88٪ لصنف امل و95.18٪ للشوفان . اظهر طيف الامتصاص للأشعة الحمراء ان جميع النماذج كان لها نفس طيف الامتصاص العائد للبيتا كلوكان مع بعض الاختلافات في مواقع وقيمة الامتصاص. لقد كان هنالك منحى امتصاص في الرقم الموجي 1304.37سم$^{-1}$ ظهر في نموذج العينة القياسية لبيتا كلوكان الشوفانى. كذلك أظهرت نتائج كرموتوكرافيا السائل فائق الاظهار النقاوة العالية للبيتا كلوكان فقد ظهر منحنى واحد مطابق لمنحنى البيتا كلوكان القياسي والذين كان لهم زمن مكوث ما بين17.429 الى 17.453 دقيقة.

الكلمات المفتاحية: بيتا كلوكان الحنطة، الشيلم، الشوفان، الشعير، كفاءة استخلاص البيتا كلوكان، كروموتوكرافيا السائل فائق الاظهار، الاشعة الحمراء
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INTRODUCTION

Cereal β -glucan (1 → 3) (1→ 4)- β-d-glucans are defined as fibrous structures found in the aleurone, Cell walls of cereals sub-aleurone and endosperm. In general, the composition of b-glucan in barley, oats, rye and wheat is 3–11%, 3–7%, 1–2%, and,1%, consistently (3). There are variations in the composition of β-glucan across cereals, with a more significant percentage identified in resources of barley, after the resource of oat, and a reduced proportion in sources of rice and wheat. Widely, β -glucan constituents that have been collected comprise more than 50 per cent of the fiber composition. Nonetheless, most aggregate contains significant concentrations of starch and proteins tied to the molecules with which enzymatic therapies are recommended to help eliminate these extracts' impurities (5). Extraction utilizing wet approaches requires inactivation of the endogenic enzymes and acid or alkaline solvent processing. Contaminant reduction (proteins and starch) is achieved with hydrolytic enzymes or by direct adsorption. B-glucan precipitation is achieved with alcohol (99 %), and eventually, the β-glucan extract procedure is done with dehydration (by freezing or spray drying)(4,7). By using wet strategies for the derivation of β-glucan, the temperature, pH, exposure time, and molecular weight should be monitored. However, even at low concentrations of β-glucan wet extraction process are characterized by a high cohesiveness of the aqueous extracts. This contributes to greater fluid quantities, and high concentrate drying expenses. Outside this, low pH and endogenic enzymes can decrease the β-glucan molar volume when subjected to humidity (6). Purification procedures for the removal of starch and proteins after extraction of β-glucan are often not conducted. Such pollutants in the extraction may inhibit proper analysis and cause considerable difficulties in food application. Enzymatic α-amylase and protease intervention (7). The overall β-glucan amount of oats was measured by using Megazyme Mixed-Linkage-β-glucan Assay Package method (Megazyme method) has been successfully evaluated by AOAC International (Method 995.16), AACC (Method 32-23.01) and ICC (Method No. 166, approved 1998), along with improvements. Through the practice of α-amylase, lichenase, and β-glucoseoxidase in the implementation of enzymatic methods (15).

The aim of this research is to study the responsibility of different cereal crops to extraction and purification of β-glucan by restricted method and effect it on their characteristics.

MATERIALS AND METHODS

Materials

Cereal grains samples of Wheat (Aras), Rye, Oat, Barley (Aksad and Amal) from local cultivar were used in this study.Megazyme kit was obtained from (megazyme company, USA) to determine β- glucan. Standard oat β-glucan was generated from megazyme with purity >94 per cent and low viscosity, Protenaise-k-(3000u / g) was acquired from Bio-lab.

Methods

Cereal grains samples were washed twice at room temperature, then cured inside the (oven,memmert,Germany)at 50°C. Eventually, a 40-mesh panel was used to mill the wholegrains. potassium phosphate buffer (20 mM, pH 6.5) was obtained by dispersing KH2 PO4.2H2o in 900 ml of distilled water, accompanied by pH adjustment with (4g/L) NaOH, adjusting volume to 1L.

Proximate Analysis

Each cereals flour was analyzed for moisture (Oven at 102°Cº) and protein (Kjeldhal process with protein N factor of 5.7) according to (AACC 44-15,46-10) procedures respectively, lipid (Soxhlet; hexane) using (AOAC2000), and β-glucan (AOAC 995.16) content, ash (combustion at 525°C) contents, total carbohydrates by differences were also determined. For each study, repeated, measurements were conducted.

Extraction of grains β-glucan

β-glucan of grains were extracted using alkaline extraction method (21) as shown in Figure 1, and crude β-glucan percent was determined according to:

crude β-glucan %= weight of extracted β glucan (g)/ weight of cereal grain (g) X 100

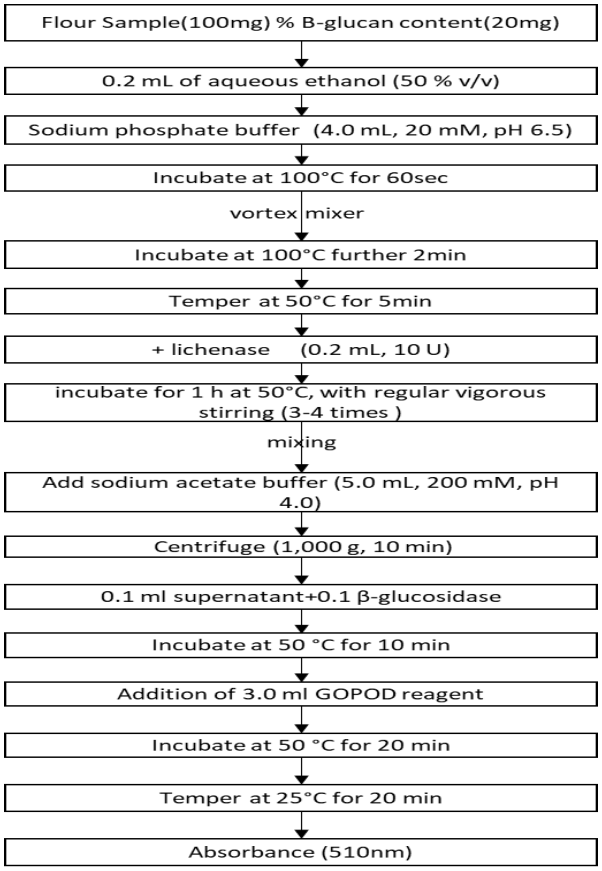

Figure 1. Extraction of grains β- glucan

Purification of β- glucan

The crude β-glucans were purified by pretreated them with the addition of Potassium phosphate buffer 1g/25ml (20mM, pH7.5), then samples were mixed with proteinase-k-5.8ml(3000u/g) placed into water bath at 40°C, 3h, then 60% ethanol was added and Centrifuged, 2000g for 10 min. The precipitate dried by hot air 60°C,6h which was done to extract free sugars and flush off specific proteins. By precipitation with aqueous ammonium sulfate (30% w/v) at 5°C for 72h as described previously by YOUNG-TACK, et al. (5) with little modification. The precipitate was redissolved in potassium phosphate buffer (20mM, pH6.5), and the solution was cooled. Dialysis membrane was used to remove the ammonium sulfate. The percentage of Purified β-glucan was calculated as follow:

Purified β-glucan% = weight of purified β glucan (g) /weight of cereal grain (g) X100

β-glucan content analysis

Total b-glucan content was determined by using a β-glucan assay kit for mixed linkage (β-glucan Assay Kit [Mixed Linkage (AACC method 32-23.01AOAC method 995.16, and ICC standard method no.168)], Megazyme, Ireland), with some alterations the β-glucan during pretreatment process the material was analyzed. Flour samples which are dry homogenized, 100 mg (80–120 mg) of each flour were taken as shown in figure (2)

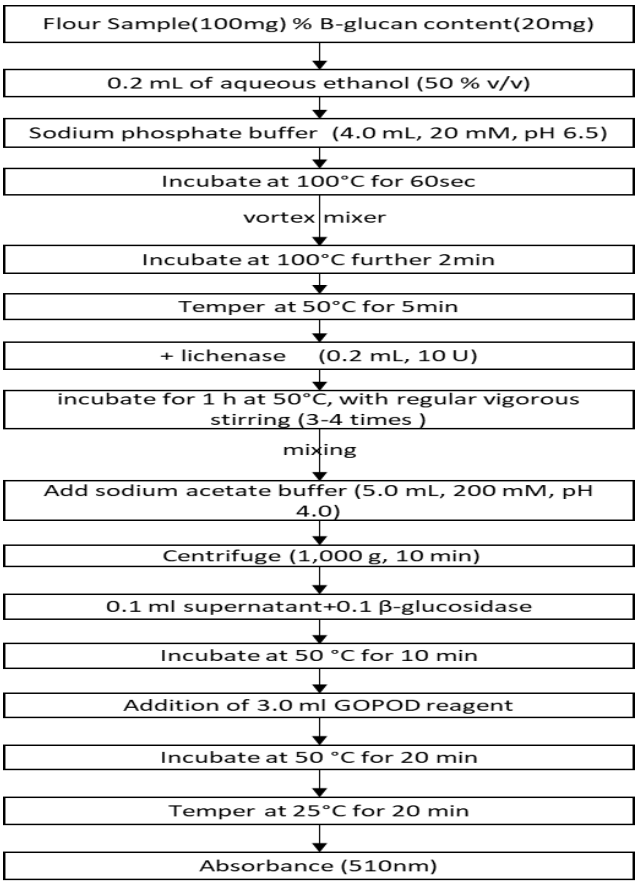

Fig2. β-glucan content analysis

Detection of ß-glucan

High-Performance liquid chromatography with detection of refractive index (RID) (Waters 2695 alliance HPLC) was used to determine the purity extracted β-glucan compared with standard medium molecular weight of oat β-glucan (imported from Megazyme.) as described previously (22).

FT-IR Spectrophotometer analysis

Fourier transform infrared spectrophotometer (FTIR), as defined formerly by Ji, et al. (23) used for the isolated polysaccharides and compared with standards to examine the structural characteristics of the -d-glucan specimens collected. The spectra were measured from 4000 to 400 cm 21 in absorbance mode (mid-infrared region).

RESUITS AND DISCUSSION

Proximate analysis of cereal crops

As shown in Table 1. moisture content of cereals flour were between (12.064-12.748%), the higher protein content was in wheat flour (12.12%) however, oat flour reported as the highest lipid content (3.905) but, also the lowest protein content related to oat flour (7.81 %). Abid &Naser's experiment(24)reported that the chemical composition of two classes of barley (IPA99 and IPA265) Flour was as following.The percentage of moisture, protein and fat in class IPA265 was higher (12.60%, 13.20%, 2.8%) respectively, than that for class IPA99 (12.05%, 11.70%, 1.50%) correspondingly. While the percentages of fiber, carbohydrates and ash were lower in the flour of class IPA265, being 5.9, 66.5 and 4.9% respectively, as compared to that of class IPA99 flour, being 6.15%, 69.4% and 5.14% respectively. Kunkulberga, et al. (25) have also shown a clear negative association (r= −0.981) between starch content and protein in grains of most common rye cultivars.

Table1.Proximate analysis of cereal grains

Cereal grains	Moisture %	Protein %	Ash %	Lipid %	Carbohydrate (by difference) %
Wheat	12.409	12.120	0.987	2.287	72.197
Oat	12.748	7.810	0.991	3.905	74.454
Rye	12.064	11.761	0.984	2.289	72.903
Barley (Aksad)	12.633	11.591	0.985	2.232	72.560
Barley (Amal)	12.598	11.640	0.974	2.224	72.564
LSD	0.440	0.332	0.090	0.515	0.001

The extractability of β-glucan from varying cereal flours: Table 2. illustrates the percentage content of β- glucans in cereal samples which indicated that rye contained the lower percentage of β- glucans (0.756±0.043%) while, both types of barley Aksad and Amal contained highest amount (9.489 ± 0.7330%) and (7.395 ± 1.0204) respectively. On the other hand, wheat and oat contained about (4.22±0.2121%) and (6.45±0.3167) respectively. Showing that there was a noteworthy alteration was shown between all the studied samples of cereal grains in their content of β-glucans. The literature reported a wide range of β-glucans content among the cereal crops. HozoVá, et al. (26) found that insoluble β- glucans, which represent about 3/4 -2/3 of the total β-glucans, ranged between 15.34 to 18.67, 5.18 to 28.2 and 8.48 to 16.09 g/100 g dry matter for barley, oat, wheat varieties respectively. These values were remarkably higher than that they reported in their research attributing these differences mainly to the enzymatic methods which determine only the water-soluble β-glucans. However, although there is a vast difference between the researchers about the content of cereal crops of β-glucans there is an agreement that barley and oat contain higher amount compared with rye and wheat which contain a lower amount of β -glucans (27).

Table 2. β-glucans content of studied cereal grains

Cereal grains	β- glucans %
Wheat	4.22±0.2121
Oat	6.45±0.3167
Rye	0.756±0.0431
Barley Aksad	9.489±0.7330
Barley Amal	7.395±1.0204
LSD	0.765

The efficiency of crude β- glucans Extraction: The amount of extractable crude β- glucans is a measure for the efficiency of the method of extraction although of presence an essential macromolecule such as polysaccharides and polypeptides may extract with it. However, the result of this work Table 3. showed that the crude extracted of β-glucans from wheat, oat, rye, barley (Aksad) and barley (Amal) were 0.373,1.409, 0.26, 2.287 and 1.855 g/100g flour respectively which may consider little amount can extract

compared with the content of each cereal crop. These obtained amount of β-glucans reflected the efficiency of extraction process which ranged between 8.83 ± 0.01for wheat to 34.39 ± 0.02 for rye, but, in general, the efficiency of extraction was higher in those cereal grains have high percentage of β-glucans such as barley and oat compared to wheat. Zhang, et al. (27) obtained a yield of 4.0% (w/w) as a crude β-glucans with 80% purity and usually, the β-glucan recovery was 82.1% (w/w) from barley flour. The purity of extracted β- glucans is important but although,Sampson et al.(28) reached to efficiency of extraction about 19.1 to 41.9% of crude β-glucans according to the genotypes of corn and isolation method but the purity of these crude extraction not exceeded more than 6.108 – 7.59%. However, the results showed there is a negative correlation between the purity and the efficiency of extraction according to the availability of isolation methods. That is mean, there are a lot of β-glucans will be lost during its isolation and purification attributing to the strong bond with other materials, especially in the cell wall (29)

Table3. Efficiency of crude b-glucans extraction

Cereal grains	Crude extracted β-glucan gm/100g flour	Efficiency of crude extracted β-glucan %	Mean of weight for final purified β- glucan mg/ 1gm of crude extract	The gain of purified β glucan from crude extracted β glucan %
Wheat	**0.373±0.027**	**8.83±0.01**	**75**	**7.50**
Oat	**1.409±0.335**	**21.84±0.20**	**216**	**21.60**
Rye	**0.26±0.0450**	**34.39±0.02**	**64**	**6.40**
Barley Aksad	**2.287±0.040**	**24.10±0.03**	**290**	**29.0**
Barley Amal	**1.855±0.0140**	**25.08±0.12**	**140**	**14.00**
LSD	**0.008**	**0.352**	**1.819**	**1.159**

Data in the table (4) represent % of β- glucan obtained from the purification of extracted β-
The results showed that these purification processes especially, the precipitating by glucan using three steps including three steps of purification.ammonium sulfate were useful for purifying of oat and barley β- glucan which their purity increased to about 10- 15% to reacht 84.88 for barley (Amal) to 95.18% for oat. Although, using ammonium sulfate increased the purity about 3-7% in wheat and rye but in the same time the final amount of purified β- glucan was minimal since most of the crude extracted of β-glucan was lost during the process of salt precipitating and dialysis (see Table 4). However, the results of this work suggest using ammonium sulfite and dialysis useless for wheat and rye β-glucan purification. Zhang, et al. (27) used alkali extraction and repeated-precipitation with ethanol to isolate β-D-glucan had high purity of (80.8%) from hull-less barley (Hordeum vulgare L. var. nudum Hook. f.).

Table 4. Percentage of β-glucan obtained from the purification

Cereal grains	β- glucan% before dialysis	β- glucan% after dialysis	β- glucan% after dialysis with alcoholic washing	Cereal grains
Wheat	**51.4±1.965**	**57.135±1.265**	**58.32±1.393**	**Wheat**
Oat	**80.95±18.85**	**94.08±10.861**	**95.18±10.917**	**Oat**
Rye	**31.21±3.592**	**34.95±3.450**	**35.55±3.599**	**Rye**
Barley Aksad	**89.47±8.541**	**95.51±8.1388**	**95.81±8.061**	**Barley Aksad**
Barley Amal	**73.07±8.131**	**84.00±10.578**	**84.88±10.245**	**Barley Amal**
LSD	**12.982**	**10.123**	**10.051**	**LSD**

Quantification of β-glucan release

Figure 3. shows the result of β-glucan release (gm/100gm) which influenced by the process of extraction and purification. The core purpose of this research was the purity more than the amount of yield. Thus, there is considerable material lost in the extraction and purification tools. However, it was noticed that using 30% of ammonium sulfate was not enough to precipitate all β-glucan from the solution of crude extracted of wheat and rye β-glucan, meaning that solution contained a considerable amount of a small molecular weight of β- glucan. In contrary, this treatment did not cause a high loss of rye and barley β-glucan. The results of barley and oat were in agreement with Irakli, et al. (20) who used alkaline solution and enzyme treatment

followed by precipitating the barley β-glucans from their solution by using 37% ammonium sulfate to obtain high purity of β- glucan (93%). As a result of AL-Esawi, et al. (10) study neutralization on the protein particles, a decrease in protein solubility and exude of water layer coated the protein particles may occur and hence particles is deposited. A relationship is found between the concentration of sulfate used in precipitation and the quantity and distribution of loads and the number of non-ionic and water-damaging aggregates on the surface of the protein molecule. Shamurad.et.,al (8) stated that the enzymatic modification which considered the most safety method to get a good functional an nutritional characteristic of wheat protein.

Quantification of β- glucan release

Table 5. showed that there were some differences and similarities between the control sample (oat's β- glucan) and purified studied cereal β- glucans samples in their values of Ft- infrared wave and maximum absorption. A board peak was recorded at a range of wave numbers from 3392.89 for rye to 3434.96 cm^{-1} for aksad β- glucan for all samples which it is ascribed to the alcoholic group O-H stretching vibration of carbohydrate. Also, Du (27) reported that the influential band at 3386 cm^{-1} was indicated to the hydroxyl stretching vibration of the extracted polysaccharide. However, the waves number 3386.67, 3402, 3417, 3430 cm^{-1} were allocated to O-H stretching vibration intermolecularly and intramolecularly in β-glucan from different sources (27). However, all the samples showed a broad curve at these wave nos. Nevertheless, they differ in their maximum absorption, which ranged from 0.4 for wheat to 1.69 for rye β- glucans. The fingerprints (1400- 500 cm^{-1}) emphasized this results by another curve ranges from 1330-1430 and 650- 770. All studied samples absorb IR wave about at 1350, 1383.95 and 1421.40 cm^{-1} also, an absorption was noticed in all studied sample at 765.04 and 660 cm^{-1} with some differences among the samples but this range of wavelengths attributes to alcoholic O-H indicating to the presence of polysaccharides. Du (27) claimed that the precise, strong points at 2934 cm^{-1} were due to the C-H stretching vibrations. The findings of this research showed that all the samples contained a sharp curve ranged from 2926.32 to 2933.93 cm^{-1} which attributed to CH_2 stretching vibration. Also, in all studied samples, an absorbance for CH_2 was showed at 1421.40 and 1383.95 cm^{-1} a week bending vibration. The result also, showed that the contaminated protein of all β - glucans samples including the control (96% oat β - glucans) absorbed the IR waves at (2147.24-2435.40 cm^{-1}) which was attributed to the protein presence by Du (27) who identified a tiny signal at 2362 cm^{-1} as indicated to the protein contamination (N-H absorbing groups). On the other hand, all samples showed absorbance at 1653.26, 1525,1450 cm^{-1} presenting amide I, amide II and amide II respectively may due to the remaining of insoluble protein with the. Although, the hydroxyl group O-H interrupted with amide II wavelengths region, generally this group belongs to the same amino acid. The IR curve of control sample (oat β- glucans) identified by contains a strong sharp peak at 1304.37 cm^{-1} in addition to not appear any other curve until 1078.97cm^{-1} while the studied samples of β- glucans did not contain peak at 1304.37 cm^{-1} but contained different peaks at this region of wavelengths (from 1304.37 to 1078.97 cm^{-1}). However, the absorption at 1304.37 may vary due to the presence of amide group that may explain the disappearing of this peak which may attribute to the protease treatment that used in the purification the studied β- glucans.

The results showed an identified peak in all studied samples, except control sample at about 1230-1250 cm^{-1} which may attribute to the polysaccharide bonds especially C-O bending . The results also showed another peak that was absented in control sample at 1158.71- 1155.85 cm-1. Zhang,et al. (27) reported that besides, sharp points at 1154 cm^{-1} and β-D-glucopyranose ring vibrations overlapping with stretching vibrations of (C-OH) side groups and (C-O-C) glycosidic bond vibrations of β-(1 → 4) and β-(1 → 3), respectively, were ascribed to 1070 cm^{-1}. Also, the peak that appears at 1080.75 cm^{-1} in all samples except control which was 1078.97 cm^{-1} may both are similar since Du (27) attributed these bands at 1082 and 1070 cm^{-1}

respectively to the C-O-C and C-O-H link band in glucopyranose ring. An identical board peak at 1019.31 to1024.00 cm^{-1} appeared in all studied samples except control (oat β- glucan) which was a small peak at about 1030 cm^{-1} and overlapped with the peak at 1078.97 cm^{-1}. It can conclude that the differences between the two methods to produce the β- glucan is the leading cause this difference since the studied oat β- glucan has differed from the control β- glucan (imported from Megazyme co.). The results showed that there were four bands of IR absorbance appeared clearly and sharply in all samples except control and barley var. Aksad. These bands were at about 931.63, 860.75, 765.22 and 680±20 cm^{-1} to the vibration of (COC), (CC), (CO), (CCH) (CO)ring, (CC)ring, (COC), (CCO), (OCO) of glyosidic bonds. The results also showed that there were two peaks at 574.86 and 527.48 cm^{-1} in all studied samples, although there is a difference among the samples in their peaks areas and absorbency. This result is with the agreement of JÄRVAN, Lukme, Tupits and Akk (6) who attributed the bands at 573 cm^{-1} to the bending vibration of polysaccharide while 523 cm^{-1} to pyranose ring C=O a symmetric deformation. Finally, it can conclude from the IR results the following:

There is shifting between the wavenumber of control (high purity of oat β-glucan) and the studied samples. This shifting may attribute to the method of extraction and purification more than on the type of cereal since this shifting was between the studied oat and oat control. Using different method may affect the intensity of hydrogen bonds, degree of crystallinity, the type of binding between β-glucan and other impurities, especially protein and pentosans. All the bands that the researches indicated to take place absorbance at these regions by polysaccharides are present in the all scanned studied samples with some differences in their areas and absorbance. A robust and sharp peak at 1304.37 cm^{-1} appeared only with the control sample while it is absent in the studied β-glucan. This band did not determine by researches as a region for β-glucan absorption but, Wiercigroch,et al. (13) claimed that the band at 1306 cm^{-1} found only in raffinose or trisaccharide as a result of CH_2 twisting vibration. On the other word, did not contain the studied samples such this band not mean there is a defect on purified β-glucan. There are somewhat differences between the two types of barley β-glucan (Aksad and Amal), especially at the region between 400-1000 cm^{-1} in, which, Aksad bands were very short and not identified compared with Amal. However, in this region where the structure of pyranose units is effective, may some changes take place due to its ability to rearrange its structure caused decreasing of its capability to absorb IR wave at these bands.

Comparison between purified β- glucan and standard oat β- glucan

In this study, HPLC was used for comparing the purity of purified cereal β-glucan with the standard (Oat β-glucan) and detection of quantification of β-glucan hydrolysis with lichenase. Table 6. and Figure 2. showed that all purified cereal β- glucan appeared only one peak at the same Retention time RT of standard oat β- glucan which ranged between 17.429 to 17.453 min that may give somewhat an indication about the purity of the prepared cereal β- glucan. Also, Abd El Ghany, et al. (14) identified peak of (1→3)(1→6)-β-D-glucan extracted from *Pediococcus parvulus* by using HPLC, had retention time at 9.044 min. The results also showed that all the purified extracted cereal β-glucan have the same RT either between the tested β-glucan or with standard β-glucan was about 17.45 min. This may mean that the extraction and purified method caused producing β-glucan has the same molecular weight as standard β-glucan, which has moderate molecular weight. The results indicated that there is a difference among the tested β-glucans in their resistance to lichinase hydrolysis depending on the time of break down may occur due to their difference in structure. β-glucan of barley (type Amal) had a higher resistance to

hydrolysis until 15 min. but it was decline completely at 20min. (as the result of the determination of β-glucan showed that.) Also, β-glucan of rye firstly at 7 min profoundly analyzed then resisted the lichinase hydrolysis for 15 min or may more time. On the other hand, β-glucan of barley (type Aksad) oat and wheat completely hydrolysis during 15 min although there was some retardation of β-glucan of oat and wheat at 7min. compared with Aksad type.

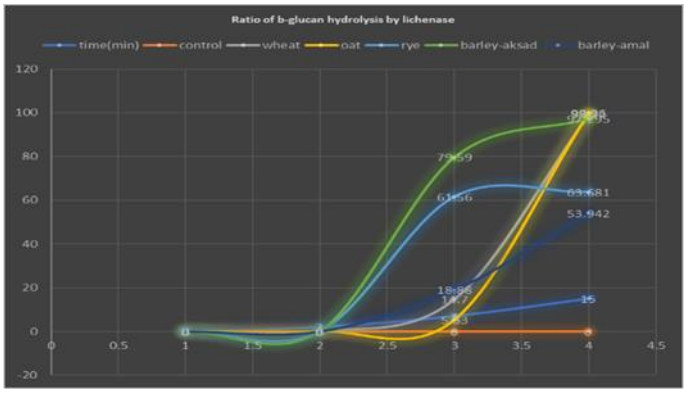

Fig3. Ratio of β- glucan hydrolysis by lichenase

Table 5. Ft-IR Spectra of cereal grains

Control (oat)		Wheat		Oat		Rye		Barley (aksad)		Barley (Amal)	
Wave number cm^{-1}	Maximum absorbance	Wave number cm-1	Maximum absorbance	Wave number cm-1	Maximum absorbance	Wave number cm-1	Maximum absorbance	Wave number cm-1	Maximum absorbance	Wave number cm-1	Maximum absorbance
3417.55	0,90	3434.73	0.40	3411.69	0.60	3392.89	1.69	3434.96	0.55	3399.84	1.52
2933.93	0.72	2928.93	0.50	2930.18	0.37	2927.22	1.30	2926.32	0.45	2929.47	1.15
2860	0.67	2147.24	0.14	2147.24	0.14	2800	1.00	2800	0.42	2147.24	0.43
2435.40	0.55	1750	0.21	1750	0.20	2147.24	0.46	2147.24	0.31	1655.13	1.15
1657.74	0.79	1653.26	0.29	1653.20	0.35	1730	0.62	1700	0.37	1541.65	0.82
1544.33	0.69	1544.33	0.22	1544.33	0.25	1656.88	1.00	1655.28	0.44	1421.40	1.00
1421.40	0.65	1421.40	0.26	1421.40	0.34	1544.33	0.69	1544.33	0.38	1350	1.00
1383.95	0.65	1383.41	0.26	1383.95	0.34	1421.40	0.88	1421.40	0.40	1325	0.95
1350	0.65	1350	0.25	1383.15	0.34	1383.95	0.88	1383.95	0.42	1240	0.92
1304.37	0.82	1230	0.25	1347	0.34	1370.40	0.92	1230	0.40	1156.19	1.52
1078.97	1.04	1158.41	0.37	1249	0.32	1230	0.82	1156.19	0.46	1080.45	1.69
1043	0.85	1080.75	0.39	1157.87	0.49	1155.85	1.52	1080.45	0.48	1020.42	1.00
1000	0.88	1019.31	0.45	1080.65	0.58	1080.45	1.69	1024.00	0.50	893	0.79
893	0.85	893	0.25	1019.45	0.64	1020.05	1.92	860	0.37	861.00	0.67
880	0,85	861.55	0.22	901.63	0.29	893	0.69	765.03	0.35	765.03	0.79
860	0.82	765.03	0.26	860.75	0.25	861.43	0.65	690	0.38	680	0.95
700	0.61	670	0.28	765.22	0.32	765.03	0.76	527.48	0.43	600	1.04
765.03	0.61	574.50	0.32	700	0.34	690	0.82	500	0.44	576.77	1.04
625	0.63	524.33		600	0.39	600	0.92			527.48	1.000
535.04	0.92	420-430		574.20	0.43	574.86	1.00			430	0.95
430	0.74			526.84	0.38	527.48	0.92				
405	0.76			450	0.32	420	0.76				

Table 6. Ratio of β-glucan hydrolysis by lechenase

Cereal β-glucan	Time of reaction with enzyme (min)	RT	Area	Injection amountμl	Area according to 10 μl	Ratio of hydrolysis by lichinase %
Control	-	17.453	11,857,690	10	11,857,690	- (Not treated)
Wheat	2	17.433	33,228,606	10	33,228,606	0
Oat	2	17.441	33,833,054	10	33,833,054	0
Rye	2	17.446	34,803,900	10	34,803,900	0
Barley (Aksad)	2	17.429	32,725,532	10	32,725,532	0
Barley (Amal)	2	17.445	35,321,400	10	35,321,400	0
Control	-	17.453	11,857,690	10	11,857,690	-
Wheat	7	17.335	28,342,556	10	28,342,556	14.70
Oat	7	17.500	32,026,844	10	32,026,844	5.33
Rye	7	17.437	13,378,293	10	13,378,293	61.56
Barley (Aksad)	7	17.373	6,678,139	10	6,678,139	79.59
Barley (Amal)	7	17.472	28,613,549	10	28,613,549	18.99
Control	-	17.453	11,857,690	10	11,857,690	-
Wheat	15	17.593	2,559,620	100	255,962	99.22
Oat	15	17.640	1,019,480	100	101.948	99.69
Rye	15	16.117	161,207,139	100	16,120,713	63.681
Barley (Aksad)	15	17.488	8,851,428	100	885,142	97.295
fiBarley (Amal)	15	16.100	162,680,549	100	16.268.054	53.942

CONCLUSION

Although, there is a difference among the studied cereal grains in their content of β-glucan but the differences of their responsibility forward extraction and purification were wide. The efficiency of beta glucan extraction depended on the content of cereal grains of it but the purity may be independed characteristic. Therefore, using a modified method of β-glucan extraction and purification which are suitable for each cereal crop may increase the efficiency of the extraction and purification. However, using Ft infrared and HPLC are very suitable to determine the beta glucan properties included the purity.

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