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Study on Estimation, Extraction and Analysis of Barley Beta-glucan

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Abstract: ***Barley is one of the most important cereal foods all over the world. It is currently used as feed for animals as well as food for human consumption. In recent years, the importance of barley grains as a nutraceutical ingredient has increased because of their high content soluble fiber, especially as a rich source of β-glucan. The most documented nutritional benefit of β-glucan is flattening postprandial glucose, insulin rise and cholesterol control. With this background the objective of the present study was framed to extract beta glucan from barley and to analyze its properties. Methodology: β-glucan content in whole barley grain flour was identified and extracted by Hot Water Extraction method. The isolated extract was further analyzed for the β-glucan content to determine the yield and recovery. Also the chemical composition (Proximate analysis), Total, Insoluble, Soluble fibre, functional properties (Water binding capacity, foaming capacity, viscosity) and biological properties (Antioxidant and Antibacterial) of the beta glucan was investigated. Results: From the study it was proved that beta-glucan has a great potential in food industry as thickener, emulsifier and functional or nutraceutical ingredient. Further extraction by hot water method could yield appreciable quantity of beta-glucan pellets with good quality.***

Keywords: Barley, β-glucan, Extraction, soluble fiber.

1. Introduction

Barley was the staple food of people in many countries for ages, but it drew away from favor due to changes in food preferences. Barley is now again returning to favor in many parts of the world for its ability to contribute to a healthy life. The high level of soluble fibre (β-glucan) in barley is reputed to reduce heart disease[1], [2], [3]; colon cancer[4]; and post-prandial glucose concentration[5]. The soluble (1,3) (1,4) β-D glucan is believed to be the barley component most responsible for this health beneficial activity[6]. The studies related to barley beta glucan were very few so the estimation and properties of beta glucan extracted from barley was tested.

1.1 Objectives

- To estimate and extract β-glucan from barley
- To analyze the properties of β-glucan isolated from barley

2. Methodology

In the present study, beta glucan was estimated, extracted and analyzed for its properties in-order to lay an idea for the food and pharmaceutical industry to utilize beta-glucan as a functional and medicinal ingredient.

2.1 Estimation of Beta-glucan

2.1.1 Selection of samples

Oats and Barley which are the common cereal source of β-glucan was selected for the estimation. Since oats is highly explored for its β-glucan content than barley, it was felt essential to identify the content of β-glucan in commercially available oats and compared with barley.

2.1.2 Procurement of Raw materials

Based on the market availability, 3 brand's of oats which are commonly available in local market and 3 forms of barley samples – whole barley grain, broken barley and pearl barley was identified and selected. Generally, Oats are available in flakes form in the market; that are imported and marketed by the national level companies. Barley was found to be available in whole and broken forms in the market; that are mostly unbranded. So, in the present work oats in flakes form and barley in whole and broken forms were selected for estimation.

2.1.3 Preparation of Samples

The oats and barley were grinded to flour by high-speed electric mill and sieved by a mesh size of 20mm.

2.2 Estimation of Beta glucan content in selected oats and barley flour

Beta glucan estimation was carried out according to the method of McCleary and Codd [7], using megazyme assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland).

2.3 Extraction of Beta-glucan

2.3.1 Selection of sample

From the previous part of the study, whole grain (De-hulled barley) was selected for the extraction of β-glucan.

2.3.2 Preparation of sample

The whole barley was milled to flour by high-speed electric mill and sieved by a mesh size of 20mm.

2.3.3 Beta-glucan Extraction

β-glucan was extracted from whole barley flour by Hot water extraction method described by AsifAhmad et al[8]. A Schematic outline of the extraction protocol is presented in fig.1.

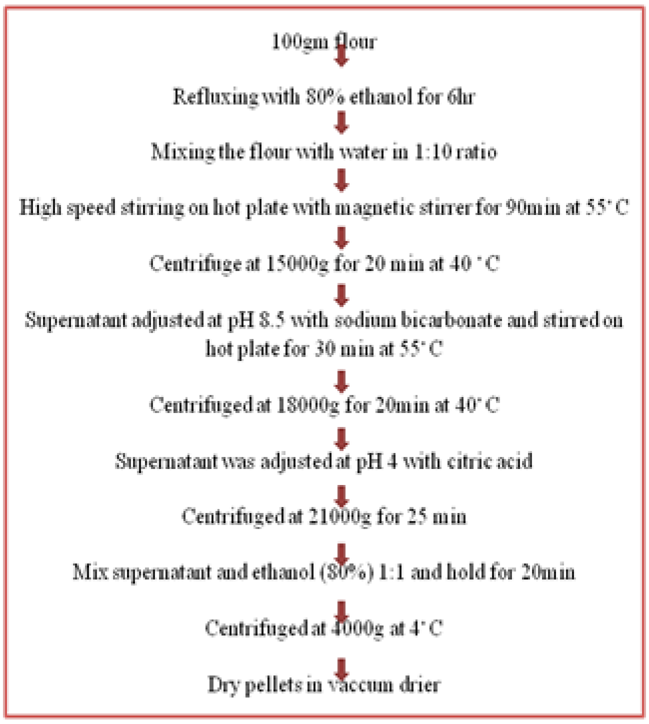

Figure 1: Procedure of beta-glucan extraction from barley

2.4 Analysis on isolated β-glucan pellets

2.4.1 Estimation of β-glucan

The isolated pellets were estimated for β-glucan content by using megazyme assay kit, Ireland. This was carried out in-order to identify the recovery percentage of β-glucan content from the barley flour by hot water extraction method.

2.4.2 Proximate composition

Crude fat and ash content were determined according to the approved methods of the American Association of Cereal Chemists Method 08-01 and method 30-10, respectively [9]. Nitrogen content was determined by the kjeldahl method [10]and converted to protein content by a factor of 6.25. Total, Soluble and Insoluble dietary fibre content was determined by following the method of IS 11062-1984.

2.5 Functional Property Analysis

2.5.1 Foaming property

The foaming capacity and foam stability (FS) were studied by following the method of Temelli[11].

2.5.2 Water binding capacity

The water binding capacity (WBC) was determined by the modified method described by Wong and Cheung [12].

2.5.3 Viscosity

Viscosity was measured using Brookfield viscometer.

2.6 Biological Properties (In-vitro)

2.6.1 Anti-oxidant activity

In the present part of the study, antioxidant activity of the isolated β-glucan pellets were identified by measuring Free radical scavenging activityand Metal chelating activity according to the method of Braca[13] and Benzie[14]. The sample taken for each analysis is 5µl and the assay was performed in three triplicates.

2.6.2 Anti-bacterial activity

Well-diffusion assay was used to evaluate the anti-bacterial activity of isolated β-glucan pellets against S.aureus (gram positive) and E.coli (gram negative) bacteria on MHA medium. Sample of different volume (10 to 40µl) at various concentrations was added into the wells. The plates were incubated for 24hours at 37˚C and the zone of inhibition was measured using zone inhibitory scale. Kanamycin (30mg) acted as control. The assays were performed in triplicates.

3. Results and Discussion

3.1 Estimation of Beta-glucan

Beta glucan is a polysaccharide that are composed of glucose units linked together by beta glycosidic linkage having (1,3) and (1,4) bonds. The most documented nutritional benefit of beta-glucan is flattening postprandial glucose, insulin and cholesterol rise. FDA has acknowledged and recommended 3gm of beta glucan per day for achieving the health benefits. As beta glucan has shown to have positive influence on human health, in the present study it was estimated in both selected oats and barley.

Table 1: Estimation of beta-glucan in Oats and Barley

Samples	*Beta-glucan (%) (w/w)*
Oats	
I	2.91
II	2.80
III	2.55
Barley	
Hulled barley	4.83
Broken barley	3.96
Pearl barley	2.95

From the estimated results, it was found barley had the maximum content of beta glucan compared to oats. This was proved by many scientists that of the common cereals like wheat, rye oats and barley, the largest amounts of β-glucan are found in barley (3-11%) and in oats (3-7%) [15]. Differences in beta glucan content among the barley were also identified. This may be due to the sample selected although barley is generally considered as the higher beta-glucan level material [16].

3.2 Extractionof Beta-glucan

The general extraction procedure of beta glucan was discussed in the methodology. The aim of this part of study is to produce beta-glucan with maximum yield and recovery. In-order to achieve this best and cheaper method was carried out and the yielded pellets were analyzed for the chemical and biological parameters to determine its efficacy.

3.3Analysis on isolated β-glucan pellets

3.3.1 Estimation of β-glucan

Table 2: Yield and percentage recovery of beta-glucan from barley

Weight of flour (gm)	*Weight of isolated Pellets (gm)*	*Percentage of Extract (%)*	
100	5.4	5.4	
Percentage of Recovery of beta-glucan content (%)			
Beta-glucan (%) (w/w)	*Name of the Sample*	*Beta-glucan content (%)*	*Percentage of Recovery (%)*
	Flour	4.83	83.8
	Extract	4.05	

From the extraction, 5.4 gm of pellets was yielded from 100gm of whole grain flour which represent 5.4% of extract. As the yielded pellets did not represent the whole quality of beta-glucan isolated the percentage of recovery was identified and it was found to be of 83.8%. This maximum recovery was achieved in the present study due to the best extraction method – hot water extraction. Study conducted by Asif Ahmad et al on different extraction procedures showed hot water extraction process had significant yield and recovery.

Previous studies by various authors [17] reported 57.8% - 88.4% by using different temperature and pH in the extraction methods. Lower efficiency by hot water extraction method was observed by Symnons and Brennan[18] that might be due to the beta-glucan cleavage by β-glucanase enzyme whereas in current study hot water method of extraction showed maximum recovery (83.8%). This may be due to the preliminary treatment involved in the extraction procedure like refluxing with 80% ethanol inactivates the β-glucanase enzyme which results in higher recovery and another reason might be due to maximum starch gelatinisation and protein solubilisation. Moreover indigenous enzyme system was inactivated.

3.3.2 Proximate composition

The chemical composition of the isolated pellets is present in the below table

Table 3: Chemical composition of beta-glucan pellet

Chemical components (%)	*Beta-glucan Pellet*
Protein	7.02
Fat	0.79
Ash	1.20
Total dietary fiber	93.81
Soluble dietary fiber	87.52
Insoluble dietary fiber	6.01

From the obtained result it was found that protein content of 7.02% in the pellets. The protein content of isolated gum may affect the various functional characteristics of the product in which they are added as a functional additive. With respect to fat content, 0.79% was obtained, this appreciable removal of fatty portion is essential, since the oxidative degradation of fat is important during processing and storage. This may produce bitter taste in the foods in which beta-glucan is added as a functional ingredient, thus the overall quality of the product can be deteriorated [19]. As concerned to ash (minerals) content of beta-glucan pellets, 1.20% was found. Detailed analysis of beta-glucan gum by Ahmad et al. showed that an appreciable amount of phosphorus (3010mg/kg) and potassium (3318mg/kg) is present in the isolated samples.

Regarding dietary fiber, major compositions of the beta-glucan consisted of soluble dietary fiber (87.52%) which is the sole component in the reduction of sugars and are responsible for reduced gycemic index [20], [21] with relatively low amount of insoluble fiber (6.01%).

3.4Functional Property Analysis

The functional properties are the intrinsic physico-chemical characteristics which affect the behavior of a food ingredient in food systems during processing, manufacturing, storage and preparation. Such functional properties are important in determining the quality of the final product [22].

Table 4: Functional analysis of beta-glucan pellet

Functional parameters	*Beta-glucan pellet*
Water-binding capacity (g/g)	3.62
Foaming capacity (%)	170
Foam stability (%)	63.10
Viscosity (cP)	50.19

The water binding capacity measures the amount water retaining capacity of the isolated pellets when it is subjected to centrifugation. This hydration property is found to be of 3.62g/g in the present study, which is higher than the water binding capacity of some dietary fibers such as wheat bran 2.6g/g, corn bran 2.5g/g and soybean bran 2.4g/g [23]. The extract with appreciable binding capacity obtained in our study could be utilized as a functional ingredient in the preparation of various food products like jam, jellies, cheese etc to overcome syneresis problem.

Foaming capacity and stability are the two important factors when the beta-glucan is used as afunctional ingredient. The above table reveals 170% of foam capacity and 63.10% of foam stability which represents high foaming capacity and stability that shows more desirable characteristics in the preparation of cakes and batter. Regarding viscosity, 50.19 cP was determined at 20°C. Different extraction procedures of varying pH and temperature may result low or high viscosity this may be due to the sensitivity of (1,3) b-D bond. Similarly the high viscosity of the beta glucan content depends on the soluble fibre than the insoluble fiber which tends to lower the viscosity.

3.5Biological Properties (In-vitro)

Biological property describes the beneficial or adverse effects of a compound on living matter. Among the various properties of chemical compounds, biological property plays a crucial role since it suggests uses of compounds in the medical applications.

3.5.1 Anti-oxidant activity

Antioxidant compounds play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk of chronic diseases including cancer and heart disease by its ability to trap free radicals in the biological system [24].

Table 5: Antioxidant activity of beta-glucan pellet

Method	*Percent of Activity*
DPPH	40.07
Metal chelating	39.22

The reactive oxygen species removal was studied in-vitro by applying free radical scavenging activity of Beta Glucan. From the result it was found that the activity on the inhibition or removal offree radical by Beta Glucan, thus exhibiting a potential scavenging role by DPPH scavenging activity of 40.07% and Metal chelating activity of 39.22%. Overall the removal of free radical by scavenging activity and bymetal binding ability of Beta-glucan makes it a super molecule-bioactive substance. The present result coincides with the study conducted by Kyoko kofuji et al[25], in which beta-glucan derived from barley extracted by various acidic to alkaline conditions proved to have high scavenging activity than various polymers that are used as food additives.

3.5.2Anti-bacterial activity

Table 6: Antibacterial activity of beta-glucan pellet

Inhibition zone Measurement (mm)						
Bacteria	*Strain - NCIM*	*Control - Kanamycin*	*Volume (µl)*			
			10	20	30	40
			Concentration (µg)			
			400	800	1200	1600
S.aureus	2079	16	4	12	19	23
E.coli	2065	21	0	6	13	19

From the results, it was found that beta-glucan extract showed inhibition at the minimum concentration of 30µl (1.2mg concentration) and at 40µl (1.6mg concentration) it demonstrated a significant inhibiting property towards gram positive bacteria (S.aureus) than the control. Whereas there was no significant inhibition towards gram negative bacteria (E.coli), minimum inhibition zone was observed at the 30µl of beta glucan extract having 1.2mg concentration. The antibacterial activity of cereal beta glucan was also proved by yun et al[26].

4. Conclusion

It was evident from the study that the β-glucan extracted by hot water extraction method has both scientific and commercial value and it is a cheap and promising additive, having a great potential with respect to its properties. Hence beta-glucan from barley could find the ability to act as a functional and nutraceutical ingredient in food products.

5. Recommendations for Future Work

The use of barley beta-glucan as a functional food ingredient definitely warrants future research. This study has generated a number of areas for further research. Particularly, beta-glucan interactions with other food components are necessary that might be able to increase its use in the development of food and other new novel products.

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