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# Extraction and Enrichment of Beta-D-glucans from different Varieties of Barley

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**Abstract—***The impact of dietary fibre on the maintainence and improvement of human health has gained tremendous interest in the last decade. Out of several cereals, barley which accounts for 12 % of the world's total cereal production possesses high amount of dietary fibre (DF) with a very high proportion of soluble viscous components. Owing to its high content of bioactive compounds, barley grain is getting renewed interest as a pivotal ingredient in the production of functional foods. The prominent functional ingredient present in cereal grains like barley or Oats is beta-D-glucan which is a non- starch polysaccharide composed of β-(1→4) linked glucose units separated every two to three units by a single β-(1→3)-linked glucose and referred to as a mixed linkage β-glucan. The FDA (Food and Drug Administration) had claimed that foods containing 0.75 g β-glucan or 1.7 g of soluble fiber per serving can reduce the risk of cardio-vascular diseases. Cereal based β-glucans are classified as soluble dietary fiber with well recognized ability for reducing blood serum LDL-cholesterol levels. Several studies and clinical findings have revealed the beneficial effects related to these cereal-derived beta-glucan isolates. Beta glucan isolates are basically the hydrocolloids with thickening properties that can enhance the nutritional value of several food products by increasing the soluble fiber content. The physiological role of β-glucans has been reported to be largely related to their ability to increase the viscocity of the gut content and modification of the absorption rates of nutrients and bile acids, moderating glycaemic response to starchy foods, lowering the serum lipid levels, especially the low-density lipoproteins (LDL) and stimulating beneficial microflora in the gut. There can be numerous ways in which this prominent soluble fiber can be extracted from different cereal grains or products like using alkali treatments such as NaOH, $Na_2 CO_3$ or using hot water extraction with thermostable α- amylase. In this work, two high beta glucan content containing barley varieties namely HBL-276(6.0 ± 0.4% β-glucan ) and Himadri-BHS-352( >5% β-glucan) have been chosen for the isolation of barley beta glucan fibre fractions. Two different methods are employed for these different varieties of barley and the fiber fractions enriched with β-glucan. The fiber fractions so obtained were analysed for β- glucan content estimation by using Mega enzyme International kit, Ireland. The analysis revealed 65.8% β-glucan for HBL-276 variety and 68.6% β-glucan content for Himadri-BHS-352. The fiber fractions so obtained were stored in high density polyethylene bags at room temperature till further analysis.*

**Keywords***: Barley, beta glucans, beta glucan extraction, beta glucan content.*

## 1. INTRODUCTION

Over the years, the impact of dietary fibre addition on the maintainence and the improvement of human health has received enormous attention. Barley, Hordeum vulgaris, one of the world's most cultivated cereal crops , accounts for 12 % of the overall cereal production in the world. has insoluble dietary fibre along with several other bio active constituents like vitamin E ( including tocotrienols), Vitamin B complex, minerals and phenolic compounds (Slavin, Marquart and Jacobs, 2000; I zydorczyk, Shahidi and Madhujith, 2006). Due to the ever increasing incidence of several chronic and degenerative diseases as well as excessive calorie intake related disorders like obesity and a close association between the intake of dietary fibre(DF) and several health benefits have enlightened the minds of consumers and by increasing their interest in the production of several food products enriched with dietary fibre ( Ooning, 2007; Slavin, 2007; Poutanen, Loaksonen, Autio, Mykkanene, and Niskanen, 2007). The prominent functional ingredient present in cereal grains like barley or Oats is beta glucans which is a non- starch polysaccharide composed of β-(1-4) linked glucose units separated every two to three units by a single β-(1-3) linked glucose and referred to as a mixed linkage β-glucan. Beta – glucans are the chief structural components of the cell walls of the barley grain and it is present in fairly good amounts in this cereal as compared to other cereals like wheat, rice etc( Lazaridou et al., 2007). It is mainly the genetic as well as environmental or the growing conditions and the association between these two factors which imparts a great influence on the beta glucan content in barley(Andersson, Elfverson, Andersson, Regner and Aman, 1999).This very nutraceutical is distributed throughout the starchy endosperm in the cell walls but it is mainly concentrated in the bran(aleurone and sub-aleurone layer fractions of the oat grain. There have been several studies reported in the past which have revealed that the beta glucan content is high in those barley genotypes which have anomalous starch composition or having high amylase in comparison to those which have normal starch. In one study it was reported that no significant differences were

found between two row and six row barley variety (Fastnaught at al. 1996). The isolation as well as purification techniques used for obtaining beta glucan fiber fractions depend on the localization of beta glucans in the barley grain and their interactions with other constituents( Fincher and Stone, 1986). In the past, various extraction processes have been evaluated and analysed as far as the beta glucan yield is concerned. There can be numerous ways in which this prominent soluble fiber can be efficiently extracted from cereal grains like barley or its products by using alkali treatments like NaOH, Na2CO3 or by using hot water extraction with thermostable α-amylase. In this study carried out, two high beta glucan content containing barley varieties namely HBL-276(6.0± 0.4% β-glucan) and Himadri-BHS-352(> 5% β-glucan) have been taken into consideration for the extraction of beta glucan fiber fractions. These fibres so obtained were analysed for their beta glucan content(assay) estimation by using Mega enzyme International Kit, Ireland. For HBL-276, the beta glucan content came out to be 65.8% where as for Himadri-BHS-352, it came out to be 68.6%.

## 2. MATERIAL AND METHODS

### Preparation of barley flour

Six rowed, good threshability and amber grain bearing prominent Indian barley variety(HBL-276) containing high amounts of beta- glucans(6.0±0.4%) was procured from SKUAST, Jammu. Barley flour was produced by grinding these barley grains through a cyclone mill(mesh size 20 mm) followed by proximate analysis. The moisture content of the barley flour was determined by Aproved method(AACC, 2000). Ash content was estimated by AOAC, 1995. Crude fat was estimated by AOAC, 1995. Protein estimation was performed by using micro-Kjeldhal apparatus. Crude fibre determination was done by AACC (2000). The beta glucan content is expressed as percent weight of the total sample using Mega- enzyme mixed linkage beta- glucan estimation kit using the method of Cleary and Glennie- Holmes (1985). The moisture content, ash, Crude protein, crude fat, crude fiber and the β- glucan content came out to be 13.4%, 2.31%, 12.6%, 2.31%, 6.57% and 5.82% respectively.

## 3. COMPOSITIONAL ANALYSIS OF BARLEY FLOUR

| Compositional analysis | Percentage |
|---|---|
| Moisture | 13.4 |
| Ash | 2.31 |
| Crude Protein | 12.6 |
| Crude fat | 2.31 |
| Crude fiber | 6.57 |
| Beta glucan | 5.82 |

## 4. CHEMICALS AND REAGENTS REQUIRED

Sodium hydroxide (1 M NaOH), Hydrochloric acid (2 M HCl), absolute ethanol( 99.99%) were procured from Sigma Aldrich Chemicals ltd. All the chemicals or the regaents required were of analytical grade.

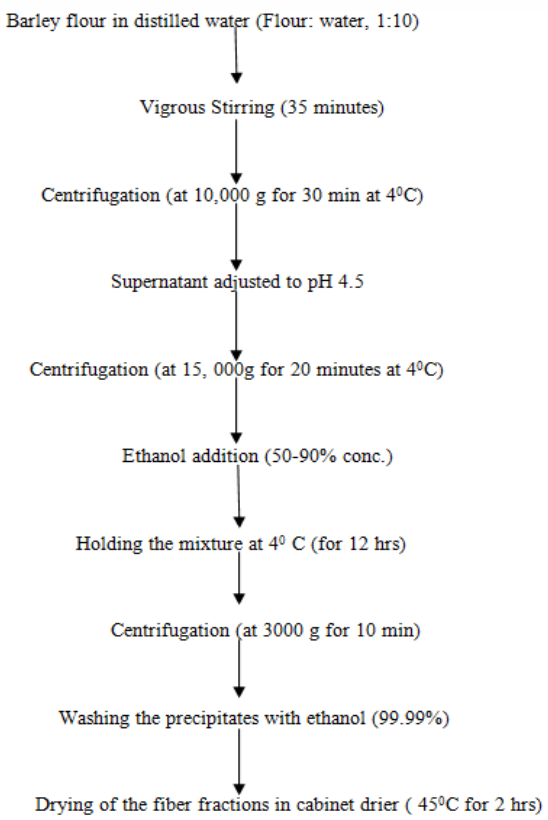
## 5. EXTRACTION AND PREAPARATION OF BARLEY BETA GLUCAN FIBRE FRACTIONS

Barley flour obtained by grinding barley grains was suspended in pre-heated distilled water ($50^0$ C) in the ratio of 1:10 followed by vigorous agitation for 35 minutes. pH of the solution was kept 7 throughout the agitation. Centrifugation of the mixture was carried out at 10,000 g for 15 minutes at $4^0$ C using refrigerated centrifuge. Supernatant was collected whereas precipitates were discarded. Addition of 2 M HCl to the supernatant to adjust the pH 4.5. Centrifugation of the solution was again carried out to separate the precipitated protein which was eventually discarded. This was followed by the addition of 99.9% ethanol for minimum ten hours or overnight at a temperature of $4^0$ C. Centrifugation was again carried out at 3300 g for ten minutes. Precipitates so recovered were subjected to homogenization with 99.99% ethanol followed by washings with ethanol only. The fiber fractions so obtained were dried in a cabinet drier for two hours.

## 6. ANALYSIS OF THE BETA GLUCAN FIBRE FRACTION

| Compositional Analysis | Percentage |
|---|---|
| Moisture | 3.5 |
| Ash | 3.16 |
| Crude Protein | 3.5 |
| Crude fat | 1.96 |
| Starch | 2.32 |
| Insoluble dietary fiber | 19.76 |
| Beta- glucan( Soluble dietary fiber) | 65.8 |

## 7. FLOWSHEET FOR THE EXRACTION OF BETA GLUCAN FIBRE FRACTION FROM BRALEY FLOUR




## 8. PREPARATION AND ANALYSIS OF BARLEY FLOUR

Another reknown six- rowed Indian barley variety having hull less grain namely, Himadri(BHS-352) was obtained from SKUAST, Jammu). Barley flour was produced by grinding these grains gain by a cyclone mill ( 20 mm mesh size) followed by proper sieving. Thereafter flour was analysed for its compositional analysis with respect to moisture, ash, crude fat, crude protein, crude fiber and as well as beta glucan assay. Moisture content determination was done by approved method (AACC, 2000) and it came out to be 13.32%. Ash content determined by AOAC, 1995 came out to be 2.52%. Crude fat and crude fiber determination was done by Approved method (AACC, 2000) and it came out to be 2.63% and 5.75% respectively. The beta glucan content estimation was done by using mixed linkage mega enzyme international kit, Ireland and it came out to be 5.23 %.

| Compositional Analysis | Percentage |
|---|---|
| Moisture | 13.32 |
| Ash | 2.52 |
| Crude fat | 2.63 |
| Crude fiber | 5.75 |
| Beta glucan | 5.23 |

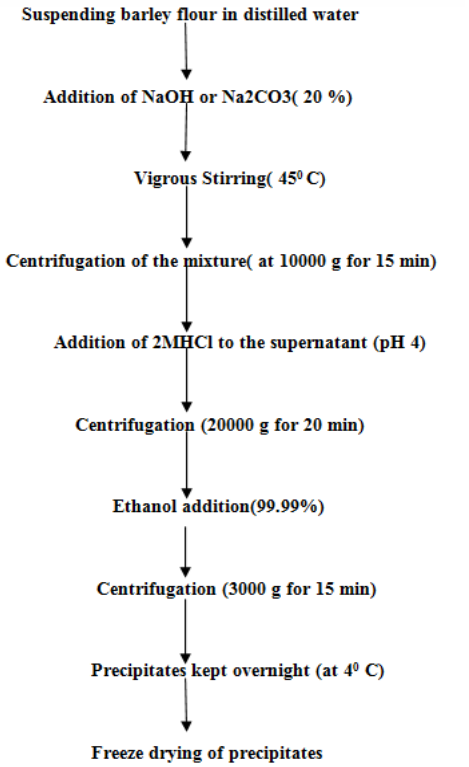
## 9. PREPARATION OF BARLE BETA GLUCAN FIBER FRACTION/ISOLATE

Suspension of the barley flour so obtained in 500 ml distilled water. pH of the mixture was adjusted to 10 by the addition of either $Na_2CO_3$ or NaOH(20 %) pellets with constant stirring for 40-45 minutes. Temperature was kept constant through the stirring at $45^0$ C. Centrifugation of the mixture was done at 10000 g at $4^0$ C for 15 minutes using a refrigerated centrifuge. Addition of 2 M HCl to the supernatant till the pH reaches 4.0. In order to remove proteins, centrifugation was again carried out at 20000 g for 15-20 minutes at $4^0$ C using refrigerated centrifuge. This was followed by the addition of pure ethanol (99.99%) in order to precipitate beta glucan fibers. Recovery of the precipitates was achieved by centrifugation at 3000 g for 15 minutes. These precipitates so obtained were kept overnight at $4^0$ C followed by freeze drying. The beta glucan fibre fractions so obtained were placed in high density polyethylene bags and kept in a cool, dark place in properly sealed condition till further analysis.

## 10. COMPOSITIONAL ANALYSIS OF BETA GLUCAN FIBER FRACTIIONS

| Compositional analysis | Percentage |
|---|---|
| Moisture | 3.92 |
| Ash | 2.23 |
| Crude protein | 5.52 |
| Crude fat | 2.96 |
| Starch | 3.09 |
| Insoluble fiber | 13.68 |
| Beta glucan( Soluble dietary fiber) | 68.6 |

## 11. FLOWSHEET FOR THE ISOLATION OF BETA GLUCAN FIBRE ISOLATES FROM BARLEY FLOU




## 12. RESULTS AND DISCUSSIONS

In this work carried out, major emphasis was laid on the yield and purity levels of beta glucans in the fiber fractions so obtained from isolating them from two different barley varieties namely, HBL-276 and Himadri(BHS-352). Yield of beta glucan fiber fractions so obtained was 80.83 percent for HBL-276 and it was 80.76 percent for Himadri-BHS-352. Purity of beta glucan in both the fiber fractions so obtained from the two barley varieties was estimated by mixed linkage Mega enzyme International Kit, Ireland and it came out to be 65.8 % and 68.6% for HBL-276 and Himadri(BHS-352) respectively.

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