EFFECT OF pH ON THE PROTEINS EXTRACTION OF BARLEY SEED COAT AND GRAINS

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Abstract

The present study was conducted to explore the effect of pH on protein extraction from barley seed coat and grains. For this purpose barley (*Hordeum vulgare* L.) seed were taken and then after grinding were separated seed coat and grains powder. The fine seed coat and grains powder were extracted in 0.1 M buffers of pH 3, 5, 7, and 10 for a maximum of 3-4 hours on the magnetic stirrer. After extraction, the extract was centrifuged at 5000 rpm for 40 minutes. Supernatant was filtered with 8µm pore size filter paper. Protein quantification was calculated by using the Bradford reagent. Protein profiles of barley seed coat and grains in different buffers extract were analyzed on SDS-PAGE under the non-reduced and reduced form. Then the appropriate statistical design was applied in this experiment. The collected data were analyzed, and inferences were drawn. The research showed that alkaline conditions were more suitable for obtaining better protein quantities. Barley seed coat showed maximum proteins at pH 7.0 and 10 which were 5.27 mg/g and 7.1 mg/g while 3.5 mg/g and 2.3 mg/g at pH 5.0 and 3.0 respectively at 25 ºC. Barley seed grains powder showed maximum proteins 8.7 mg/g at pH 10.0. 6.3 mg/g at 7.0, 5.7 mg/g at 5.0 and 3.7 mg/g at 3.0 at 25 ºC. On SDS-PAGE in barley seed coat, more protein bands were observed (10-46 kDa) at pH 10.0 during non-reduced conditions while more protein bands were observed (10-48 kDa) at pH 7.0 during reduced conditions. Barley seed grains showed maximum proteins at pH 3.0 (20-150 kDa) during non-reduced conditions while at pH 7.0 maximum proteins (10-120 kDa) during reduced conditions at 25ºC. Free amino acid estimation determined at 25 ºC both in barley seed coat and in barley seed grains. Barley seed grains showed more concentration of free amino acids as compared to seed grains which are 12.10 mg/g, 14.86 mg/g, 17.04 mg/g and 14.67 mg/g at pH 3.0, 5.0, 7.0 and 10.0 respectively. Barley seed coat showed 6.81 mg/g, 10.54 mg/g, 13.20 mg/g and 13.83 mg/g free amino acids at pH 3.0, 5.0, 7.0 and 10.0 respectively.

Key Words: Barley seeds, Bovine serum albumin, Sodium-dodecyl-sulphate, , Temperature

Introduction

Barley (*Hordeum vulgare*), belongs to the family of grasses. It is the main cereal grain that is grown in temperate climates all around the world. Barley is used as animal fodder, provide fermentable material for distilled beverages and beer and as a component of many healthy foods. It is also used in stews and soups. Barley seeds also contain some essential nutrients like protein, vitamins, dietary fiber, niacin and many dietary minerals (Zohary and Hopf, 2000 and Andersson *et al*., 2008). The seeds of various cereal plants provide a chief source of energy as well as protein in the food of man and other domestic animals. In the storage proteins of cereal seeds the low quantity of essential amino acids e.g. lysine, limits their nutritional value. Hypothetically the quality of protein can be improved by altering the composition of the seed proteins either by decreasing the components that are poor in lysine and increasing those which are rich in lysine. Cereal seed proteins play a prime role in the industry of brewing and it is source nutrition for human and animal. Barley outer layers have more protein as compare to endosperm (Yeung and Vasanthan, 2001).

By increasing the concentration of NaCl and temperature the efficiency of protein extraction was enhanced. This was probably due to increased globulin solubility. From barley, PGF hordein was extracted by using ethanol (Wang *et al.,* 2010). At 23 °C for 0.5 hr using 11.5 alkaline solution maximum extraction efficiency achieved were 74.8 %, 64.2 % and 44.8 % for pearled grain flour (PGF) of barley proteins, pearled flour (PF) proteins and Glutelin fractions. Proteins contents were 82.5 %, 79.0 % and 84.5 % respectively by (Wang *et al*., 2010). 27 % pearling degree allows removal of most outer layer proteins of barley because it contains more proteins then endosperms (Yeung and Vasanthan, 2001). Extraction efficiency significantly enhanced by an increase in pH from 10 to 11 ($P < 0.05$). A high concentration of alkali helps to break the hydrogen bonds and it detach hydrogen from sulphate and carbolic groups (Shen *et al.,* 2008). The increase in surface charge of the molecules of protein resulted in enhanced solubility of it in the solvent system. In general protein content was increased with pH, particularly when the pH was increased from 10 to 11. The maximum values of EE were reached are 45, 75, and 64 % with the protein contents of 85, 83, and 79 % for glutelin, PGF protein, and PF protein fractions, respectively, at pH 11. More harsh conditions i.e ($pH \geq 12$) was not tried in this study because undesirable side reactions like Maillard reaction might be produced between proteins molecules and polysaccharides. Proteins molecules may be denatured, starch

components may be destroyed, and increase in quantity of non-protein components can be observed under such conditions that can reduce the quality of isolates (Apinunjarupong *et al.,* 2009).

For barley protein extraction optimal pH value 11.5 was selected. It was predicted that high temperature can increase the yield of protein extraction. However, by an increase in temperature to >23 °C extraction efficiency as well as protein content decreased. This may be due to an increase in interactions between the protein and other phenolic compounds or between protein molecules and polysaccharides at high temperature and alkaline conditions. In general protein extraction from both barley PGF and PF in alkaline medium is an appropriate and effective method. The standard extraction condition was pH 11.5 at 23 °C for 30 minutes. Nearly all bands in SDS-PAGE in hordein and glutelin components appear in the endosperm fraction pattern, showing that the alkaline solution was strong enough to extract both hordein and glutelin from barley PGF. The SDS-PAGE array of barley PF protein exhibits bands at MW of 85,000-90.000, 55,000-60,000, 35,000-45,000, 25,000, and < 20,000, respectively (Wang *et al.,*2010). PGF protein extracts that was obtained by using the alkaline method had greater quantity of glutamic acid (glutamine, 26.7 %) and proline (16.6 %) as compared to the glutelin content but less than the hordein. Low quantity of lysine (1.8 %) and cysteine (1.3 %) were also obtained. This further proved that protein content obtained from barley PGF through alkaline method was mainly composed of both glutelin and hordein (Wang *et al.,* 2010).

Barley PF proteins consist of a greater fraction of threonine (4.5 %), lysine (5.0 %), valine (8.0 %), and methionine (1.7 %), but a lower quantity of glutamic acid (15.0 %), phenylalanine (3.5 %), and proline (8.5 %) as compared to endospermic proteins. Lysine act as limiting amino acid in the cereal proteins that's why the relatively high fraction of lysine in barley PF may increase its nutritive application as an ingredient of food and feed products. All isolated fractions of barley protein exhibited a balanced ratio of nonpolar and polar amino acids (Wang *et al.,* 2010).

Viscous and soft dough was formed when hordein was dispersed into pH 3-8 solution, which reduced the surface area of hordein. A significant increase in the ($P < 0.05$) solubility was noticed at pH 10, at which the structure of dough was disrupted slowly (Wang *et al.,* 2010) . Same solubility pattern was detected for the glutelin and PGF protein contents, the least values were obtained at pH 5. When pH differed from pH 5, solubility gradually increased. A clear increase in the solubility of protein was noticed at pH 10.It was anticipated that barley PF protein portion would show better solubility due to presence of soluble globulin and albumin proteins. Significant increase in solubility occurred at pH 10, as with all other fractions. The salt addition did not increase solubility. During the alkaline extraction low solubility was due to partial protein denaturation. It may be due to the binding of phenolic compounds with protein molecules, which has been described to decrease protein solubility (Viljanen *et al.,* 2005).

The values of protein solubility were usually higher when distilled water was used at pH 10 and 11 than the other pH values. Minimum solubility values in distilled water were obtained at pH 4. However protein solubility at pH 4 and pH 6 were almost similar (Yalcin and Celik, 2007). By using ethanol extraction efficiency of barley Hordein is estimated to be nearly 63 to 81 %. The most recognizable and feasible method is the use of alkaline solution for the protein extraction by (Shen *et al*., 2008). In different conditions, the extraction efficiencies varied. At pH ≤ 10 low extraction efficiency values (9 to 27 %) were obtained. When pH was raised from 10 to 11 ($P < 0.05$) extraction efficiency increased significantly. High alkali breaks the hydrogen bonds of proteins by (Shen *et al*., 2008).When pH raised from 10-11 increase in the protein contents was observed. Maximum extraction efficiency (EE) value reached were 45, 75 and 64 % with the proteins contents of 85, 83 and 79 % for glutelin, pearled grain flour (PGF) proteins and pearling flour (PF) proteins fraction respectively at pH 11.5 by (Wang *et al*., 2010). In determining the protein contents and extraction efficiency pH played an important role. Using pH 11.5 alkaline solution at 23 °C for 0.5 hrs maximum extraction efficiency was achieved as 74.8 %, 64.2 % and 44.8 % for barley pearled grain flour (PGF) proteins, pearled flour (PF) proteins and glutelin fractions respectively by (Wang *et al*., 2010).

Materials and Method

The whole experimental work was done in Institute of molecular biology and Biotechnology uol Lahore. The seed of Barley was purchased from seed market of Lahore.

Preparation of samples: 1 gram of barley seed grain flour and 1 gram barley seed coat flour were dissolved in 10 mL buffer solutions separately having pH 3.0, 5.0, 7.0 and 10.0. Barley seed grain flour and seed coat flour were stirred for inimum 3 hour on an electric stirrer. Poured all samples (4 of barley seed grain flour and 4 of seed coat flour) in each 8 falcons tubes for centrifugation at 5000 rpm for 45 minutes. Then filtrate was saved and residues were discarded.

Preparation of samples for sds-page analysis: In 4 eppendroff tubes 10 µL reducing dye solutions were added with 30 µL samples solution of barley seed grains flour also in others 4 eppendroff tubes

10 µL non reducing dye solutions were added with 30 µL samples solution of barley seed grain flour. Same process repeated for barley seed coat flour samples.

Proteins profiling by using sds-page: All samples with quantity of 15 µL were applied and run on SDS-PAGE with protein marker of thermoscientific company. At the end all proteins bands in reduced and non reduced conditions were observed and analyzed by comparing with protein marker of thermoscientific company of 26614 code. Both gels of barley seed grains flour and barley seed coat flour were stained for 30 min and destained for 45 min. Gel images were then scanned by the scanner for further analysis and labeling.

Protein quantification by bradford regent and bsa curve formation: For BSA curve and quantification of proteins dilutions made. BSA dissolved in 40 µL distilled water, then Bradford reagents 2ml added. Absorption taken by spectrophotometer (UV/Vis HALO SB-10) BSA curve drawn. Dilutions also made for both barley seed grains flour of pH 3.0, 5.0, 7.0 and 10.0 using 40 ul sample volume and also for barley seed coat flour of pH 3.0, 5.0, 7.0 and 10.0 using 40 ul sample volume by adding 2 ml Bradford regent in each sample. Absorption taken by using a spectrophotometer. Proteins were quantified in both barley seed grains flour and barley seed coat flour

estimation of total free amino acids in barley seed grains and seed coat: The complete or total quantity of free Amino acids in barley seed grains flour having pH of 3.0, pH 5.0, pH 7.0 and pH 10.0 buffer solutions also in barley seed coat flour with pH 3.0, 5.0, 7.0 and 10.0 buffer solutions checked as the technique developed by Hamilton and Van Slyke in 1943. In a test tube 5ml distillate from every sample and 5 mL 10 % pyridine was added and 25 % of Ninhydrin was also poured into the tubes. The chemical reagents in the test tubes were granted to react for the half an hour (30 min.) at 80 to 90 °C in a boiling water bath in the lab. All the mixtures were cool down and diluted further up to the 50 ml volume. With the help of spectrophotometer (UV/Vis spectrophotometer HALO SB-10) the absorbance of all samples were read and taken out at 570 nm. Total or complete amino acids in the samples were checked out by using this formula.

Total free amino acid (Ninhydrin method) was estimated by the method of Moore and Stein (1948). 1 mL of the sample was mixed with 1 mL of Ninhydrin and kept in a boiling water bath for 20 minutes. Added 5 mL of diluent (equal volume of water and propanol)and incubated at room temperature for 15 min. The absorbance was read at 570 nm against a blank reagent. The estimation was done in triplicates and the results were expressed as mg/g sample

Results

Analysis of crude protein: The total protein profile of barley seed coat and seed grains was studied. Protein extraction was done at room temperature at 25 °C and at four different pH (3.0, 5.0, 7.0, 10.0). Total proteins quantification was done by performing Bradford reagent assay and total number of individual protein bands was observed on the SDS-PAGE. Total free amino acids were also determined both in barley seed coat and in seed grains by the method of Hamilton and Van Slyke in 1943.

total protein of barley seed coat at different ph: Barley seed coat has maximum protein at pH 7.0 and 10 as shown by the figure 1. Total protein is indicated as mg/g of seed coat. Maximum protein 7.1 and 5.27 mg/g was extracted at pH 10.0 and pH 7.0 respectively.

total protein of barley seed grains at different ph: *Barley seed grains* have maximum protein values at pH 10 and 7.0 as designated by the figure 2. Maximum protein 8.7 mg/g has been extracted at pH 10. Small amount of protein 5.72 and 3.70 mg/g has been extracted at pH 5.0 and pH 3.0. It shows that basic pH conditions are favorable for the proteins extraction from Barley seed grains.

Proteins comparison of barley seed coat and seed grains at room temperature at 25°c: A total protein comparison of the Barley seed grains and seed coat at four different pH values (3.0, 5.0, 7.0, 10.0) is indicated in figure 3. It shows that barley seed grains and seed coat have almost similar concentration of protein. At four different pH, there is an increase in protein concentration from acidic pH to basic pH in both seed grains and seed coat. 8.7 mg/g of seed grains protein is maximum at pH 10 which is much higher than 7.1 mg/g of seed coat protein at pH 10. After this, second higher concentration of protein was obtained at pH 7 from Barley seed grains. Small amount of protein concentration have been seen at pH 3.0 in both Barley seed grains and seed coat

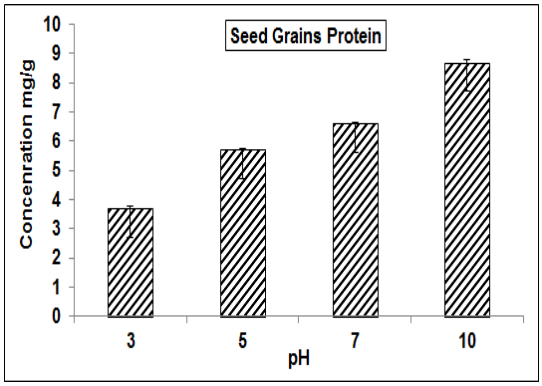


Fig. 01

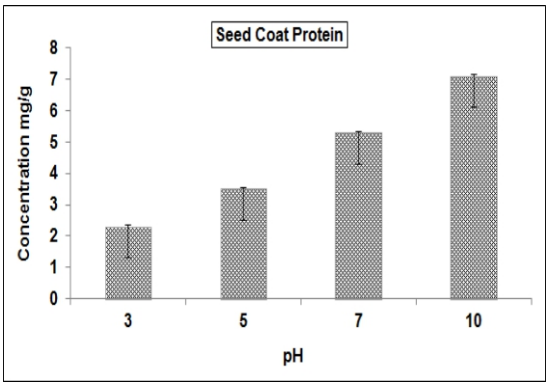


Fig. 02

Fig. 1 : Total protein quantity (mg/g) of Barley seed coat at different pH
Fig. 2 : Total protein quantity (mg/g) of Barley seed grains at different pH

Proteins comparison of barley seed coat and seed grains at room temperature at 25°C: A total protein comparison of the Barley seed grains and seed coat at four different pH values (3.0, 5.0, 7.0, 10.0) is indicated in figure 3. It shows that barley seed grains and seed coat have almost similar concentration of protein. At four different pH, there is an increase in protein concentration from acidic pH to basic pH in both seed grains and seed coat. 8.7 mg/g of seed grains protein is maximum at pH 10 which is much higher than 7.1 mg/g of seed coat protein at pH 10. After this, second higher concentration of protein was obtained at pH 7 from Barley seed grains. Small amount of protein concentration have been seen at pH 3.0 in both Barley seed grains and seed coat

Protein profile of barley on SDS-PAGE: The samples were loaded on the SDS-PAGE after calculating the protein quantification by calorimetric assay. Protein profile analysis through gel helped us to find out the molecular weight, disulphide linkage in different proteins and total number of proteins bands. Under both reduced and non-reduced condition barley proteins samples were run on the gels. Samples which were in reduced forms were heated for two minutes at 95 °C by adding β-mercaptoethanol was loaded to loading dye solution. Both gels of barley seed coat proteins and barley seed grains proteins are processed through strainer and detainer which make the gels very clear and transparent. Based on molecular weight the proteins band are fixed on the gels.

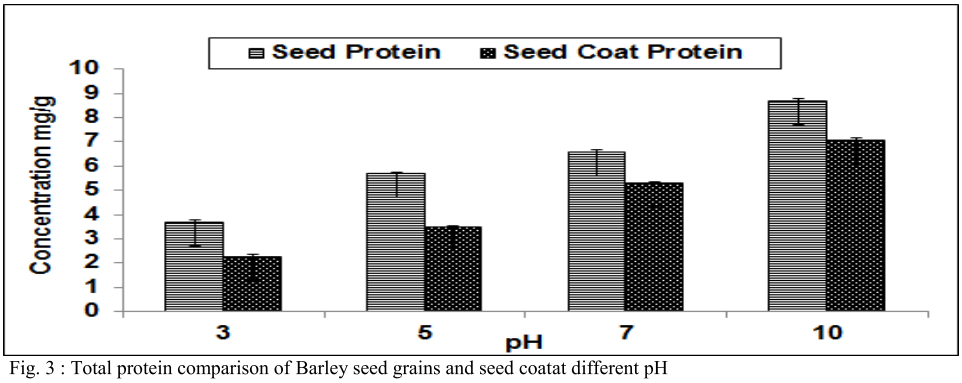


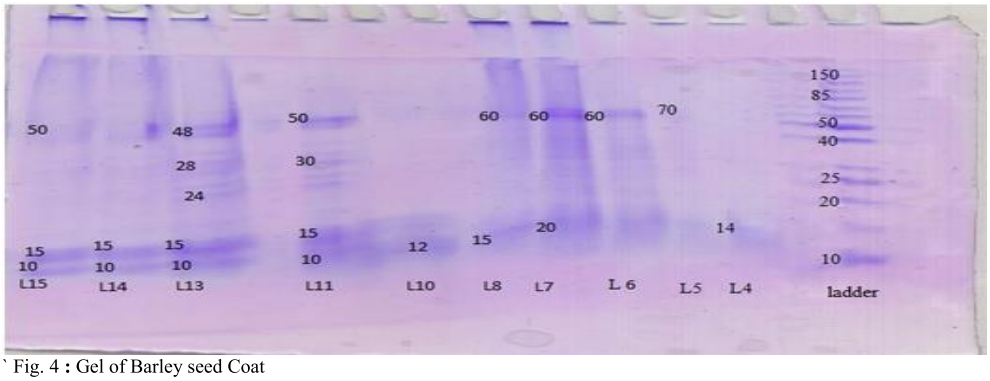
Fig. 3 : Total protein comparison of Barley seed grains and seed coatat different pH

Protein profile of barley seed coat at different pH after the gel analysis: On the 12 % SDS-PAGE all the four samples of barley seed coat proteins in reduced and non-reduced forms were loaded on the gel. Loading buffer of 4X was used. At 90 voltages gel was run for 2 to 3 hours. For better comparison of protein a single gel of barley seed coat contains both reduced and non-reduced protein profile bands. For every sample the gel loading plan is as follows.

Comparison of protein bands under non-reduced conditions: At pH 3 in lane 4 only one band is visible which is seemed to be 14 kDa. Lane 5 was also of pH 3 of non-reducing proteins sample contain only single band of 70 kDa.In lane 6 of pH 5 also single band of protein is visible and supposed to be 60 kDa while other looks blurred and broken. In lane 7 of pH 7 only two proteins bands are visible which are seemed to be of 20 kDa and 60 kDa. In lane no. 8 of pH 10.0 two bands are visible of 15 kDa and 60 kDa.

Comparison of proteins bands under reduced conditions: Lane 10 of pH 3 sample shows protein of about 12 kDa while others are clearly observed. Lane 11 of pH 5 of protein sample shows four proteins bands of 10 kDa, 15 kDa, 30 kDa and 50 kDa while others looks blurred. Lane13 of pH 7 of protein samples showing five proteins bands which are of 10 kDa, 15 kDa, 24 kDa, 28 kDa and 48 kDa others are not very clear. Lane 14 and Lane 15 both of pH 10 proteins samples which contains protein bands of 10 kDa, 15 kDa and 50 kDa.

Protein profile of barley seed grains at different ph after the gel analysis: On the 12 % SDS-PAGE all the four samples of barley seed grains proteins in reduced and non-reduced forms were loaded on the gel. Loading buffer of 4X was used. At 90 voltage gel was run for 2 to 3 hours. For better comparison of protein a single gel of barley seed grains contains both reduced and nonreduced protein profile bands.For every sample the gel loading plan is as follows**:**



` Fig. 4 **:** Gel of Barley seed Coat

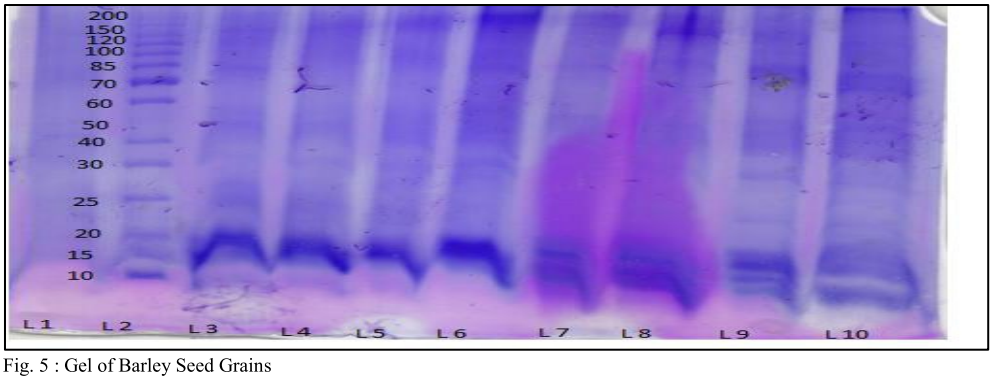


Fig. 5 : Gel of Barley Seed Grains

Comparison of protein bands under non-reduced conditions: In lane no. 2 of gel at pH 3 of protein sample five protein bands are visible of 20 kDa, 25 kDa, 85 kDa, 120 kDa and 150 kDa. In lane no 3 on the gel at pH 5 of protein sample only two protein bands are visible of 15 kDa and 150 kDa. In lane no

4 of gel at pH 7 of protein sample three protein bands are visible of 15 kDa, 100 kDa and 150 kDa. In lane no 5 of gel at pH 10 of protein sample four protein bands are visible of 20 kDa, 85 kDa, 150 kDa and 200 kDa approximately.

Comparison of protein bands under reduced conditions: In lane no. 6 on the gel at pH 3, three protein bands are visible of 10 kDa, 20 kDa and 150 kDa. In lane no 7 on the gel at pH 5 of protein sample also three protein bands are visible of 10 kDa, 15 kDa and 120 kDa. In lane no 8 on the gel at pH 7 of the protein sample five protein bands are visible of 10 kDa, 15 kDa, 20 kDa, 85 kDa and 120 kDa. In lane no 8 of the gel at pH 10 of the protein sample three protein bands are visible of 10 kDa, 20 kDa and 85 kDa. 10 kDa protein band appear in all four samples of pHs. 20 kDa protein band appear in pH 3, pH 7 and pH 10 of protein sample. 15 kDa protein band appear in pH 5 and pH 7 protein sample. 120 kDa protein bands appear in pH 5 and pH 7 protein samples.

Determination of total free amino acids: The amino acid content from Barley seed grains and seed coat was determined by using Hamilton and Van Slyke method. Quantitative determination of free amino acids in Barley seed grains and seed coat were indicated in Table 4.13. Significantly high content 14.86 mg/g and 14.67 mg/g of free amino acid were obtained in Barely seed grains at pH 5.0 and10.0 respectively followed by seed coat. Whereas, significantly lower amino acid content 12.10 mg/g and 6.81 mg/gwas observed in Barley seed grains and seed coat respectively at pH 3.0 in figure 6.

Discussion

Barley (*Hordeum vulgare* L.), belongs to the family of grasses. Barley is used as animal fodder, provide fermentable material for distilled beverages and beer and as a component of many health foods. Barley seeds also contain some essential nutrients like protein, vitamins, dietary fiber, niacin and many dietary minerals shown by the work of (Zohary and Hopf, 2000; Andersson *et al*., 2008). Seeds of many plants are the rich source of energy and proteins. Barley outer layers have more protein as compare to endosperm by the work of (Yeung and Vasanthan, 2001).

In the present study total protein profile of barley seed coat proteins and barley seed grains were studied. At four types of pH (3.0, 5.0, 7.0 and 10.0) protein extraction was done at room temperature at 25 °C. Also with the help of calorimetric assay of Bradford reagent total protein quantification was done and with reduced and non-reduced conditions. Total number of individual protein bands was observed on the SDS-PAGE. Total free amino acids were also determined both in barley seed coat and in barley seed grains by the method of Hamilton and Van Slyke in 1943.

The present study showed that Barley seed coat has maximum protein at pH 7.0 and at pH 10.0 as well as maximum protein 7.1 and 5.27 mg/g was extracted at pH 10.0 and pH 7.0 respectively. Barley seed grains have maximum protein 8.7 mg/g values at pH 10 and small amount of protein 5.72 and 3.70 mg/g has been extracted at pH 5.0 and pH 3.0 respectively .It shows that basic pH conditions are favorable for the proteins extraction from barley seed grains.

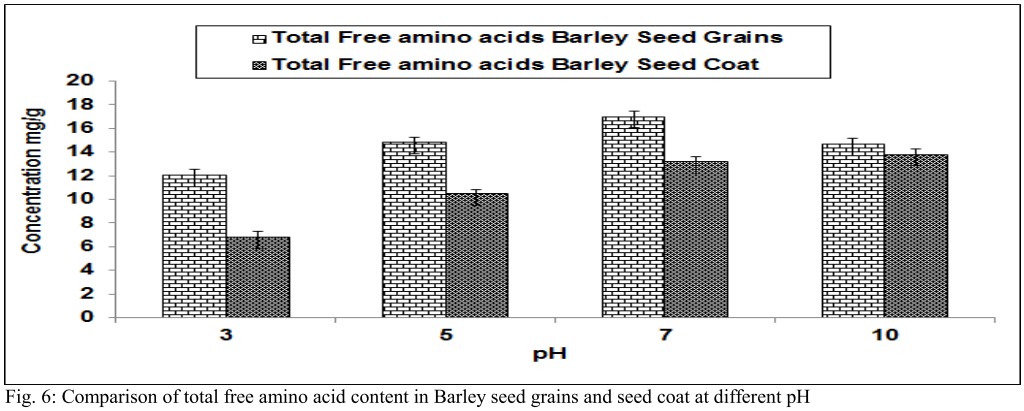


Fig. 6: Comparison of total free amino acid content in Barley seed grains and seed coat at different pH

A total protein comparison of the Barley seed grains and seed coat at four different pH values (3.0, 5.0, 7.0, 10.0) is indicated, it shows that barley seed grains and seed coat have almost similar concentration of protein. At four different pH, there is an increase in protein concentration from acidic pH to basic pH in both seed grains and seed coat, 8.7 mg/g of seed grains protein is maximum at pH 10 which is much higher than 7.1 mg/g of seed coat protein at pH 10. After this, second higher

concentration of protein was obtained at pH 7 from Barley seed grains. Small amount of protein concentration have been seen at pH 3.0 in both Barley seed grains and seed coat.

Shen *et al*. (2008) reported that in different conditions the extraction efficiencies varied. At pH ≤ 10 low extraction efficiency values (9 to 27 %) were obtained. When pH was raised from 10 to 11 ($P < 0.05$) extraction efficiency increased significantly. Wang *et al*. (2010) has observed the effect of increasing the temperature and NaCl concentration increased the efficiency of protein extraction may be due to enhanced globulin solubility. By considering the Hordein accounts for 35 to 45 % of total proteins in barley pearled grain flour (PGF) work shown by (Celus *et al*., 2006).

In the present study the protein profile of barley seed coat proteins at 25 °C after the gel analysis was studied as well as comparison of bands of the proteins under non reduced conditions was also analyzed. Under non-reduced conditions at pH 3 only one band is visible which is seemed to be 14 kDA also band of 70 kDa. At pH 5 also single band of protein is visible and supposed to be 60 kDa,At pH 7 only two proteins bands are visible which are seemed to be of 20 kDa and 60 kDa,At pH 10.0 two bands are visible of 15 kDa and 60 kDa.

Comparison of bands of the proteins under reduced conditions was also studied. At pH 3 sample shows protein of about 12 kDa, At pH 5 of protein sample shows four proteins bands of 10 kDa, 15 kDa, 30 kDa and 50 kDa, At pH 7 of protein samples showing five proteins bands which are of 10 kDa, 15 kDa, 24 kDa, 28 kDa and 48 kDa, At pH 10 proteins samples which contains protein bands of 10 kDa, 15 kDa and 50 kDa.

Protein profile of barley seed grains proteins at 25 °C after the gel analysis was studied in present study.Comparison of bands of the proteins under non reduced conditions. At pH 3 of protein sample five protein bands are visible of 20 kDa, 25 kDa, 85 kDa, 120 kDa and 150 kDa. At pH 5 of protein sample only two protein bands are visible of 15 kDa and 150 kDa. At pH 7 of protein sample three protein bands are visible of 15 kDa, 100 kDa and 150 kDa. At pH 10 of protein sample four protein bands are visible of 20 kDa, 85 kDa, 150 kDa and 200 kDa approximately.

In the present study comparison of bands of the proteins under reduced conditions also studied. At pH 3 of protein sample three protein bands are visible of 10 kDa, 20 kDa and 150 kDa. At pH 5 of protein sample also three protein bands are visible of 10 kDa, 15 kDa and 120 kDa. At pH 7 of the protein sample five protein bands are visible of 10 kDa, 15 kDa, 20 kDa, 85 kDa and 120 kDa. At pH 10 of the protein sample three protein bands are visible of 10 kDa, 20 kDa and 85 kDa.

From the barley flour with 0.1 M monothioglycerol a 39 kDa protein has been extracted at pH 5.0 and purified by ion exchange and molecular sieve chromatography and by (NH_4) SO4precipitation. It is present in at least two molecular forms of isoelectric points 5.18 and 5.22. It is an N-terminal blocked non-glycosylated single chain protein. Its amino acid composition and partial sequence analysis reveals a close relationship to barley endosperm Z protein which belongs to serpin superfamily observed by (Burhenne *et al*., 2003).

In accordance with the results of (Bindschedler *et al*., 2009), SDS-PAGE of glycogen precipitate showed substantial amount of protein of molecular weight (WM) of 20,000 to 22,000, From the extract of barley glycogen did not precipitate the molecular weight (MW) 21,000 proteins, although this protein is present in large quantity in the barley. Gel assay allowed us to purify unstudied 30 kDa cysteinendoproteinase. Two cysteineproteinaseshave been purified from the germinated barley so far by the (Jones, 2005), Also two more form of the Gibberellic acid (GA 3) induced barley aleurone layers observed by (Rayorath*et al*., 2008). The 43.7 kDa cysteine endo-proteinase purified by Bethune *et al*., 2006 EP-B cysteine endoproteinase was apparently the same enzyme by (Schmitt and Budde 2007).

In the present study total free amino acids in barley seed coat and seed grains also calculated. The amino acid content from Barley seed grains and seed coat was determined by using Hamilton and Van Slyke method. Significantly high content 14.86 mg/g and 14.67 mg/g of free amino acid were obtained in Barely seed grains at pH 5.0 and 10.0 respectively followed by seed coat. Whereas, significantly lower amino acid content 12.10 mg/g and 6.81mg/g was observed in Barley seed grains and seed coat respectively at pH 3.0,In barley seed grains the amount of total free amino acids was found at pH 3.0 is 12.10 mg/g, at pH 5.0 was 14.86 mg/g, at pH 7.0 was 17.04 mg/g and at pH 10.0 was 14.67 mg/g while in barley seed coat at pH 3.0 is 6.81 mg/g, at pH 5.0 was 10.54 mg/g, at pH 7.0 was 13.20 mg/g and at pH 10.0 was 13.83 mg/g.

By the alkali method pearling grain flour (PGF) of barley protein extract obtained had a greater glutamic acid (26.7 %) and proline (16.6 %) content compared to the glutelin fraction but less than hordein fraction. Low cysteine (1.3 %) and lysine (1.8 %) were also observed. This further confirmed that the protein fraction extracted from barley pearling grain flour (PGF) by alkaline method was composed of both glutelin and hordein work done by (Wang *et al*., 2010).

Pearled flour (PF) of barley proteins have greater proportion of proline 8.5 %, glutamic acid 15.0 %, lower proportion of phenylalanine 3.5 %, valine 8.0 %, methionine 1.7 %, threonine 4.5 % and lysine 5.0 % compared to endosperm proteins. All quantified barley proteins fractions showed a balanced ratio of nonpolar and polar amino acids

observed and analyzed by the work of (Wang *et al*., 2010).

Conclusion

In a nutshell, effet of pH on proteins extacted from the barley seed coat and grains were investigated. It is inferred that alkaline conditions were more suitable for obtaining better protein quantities. Barley seed coat showed maximum proteins at pH 7.0 and 10 at 25 ºC. Whilst in SDS-PAGE of barley seed coat, more protein bands were observed at pH 10.0 and 7.0 during non-reduced and reduced conditions respectively. Moreover, free amino acid estimation determined at 25 ºC both in barley seed coat and in barley seed grains.

Acknowledgement
This work was financially funded by "Institute of Molecular Biology and Biotechnology (IMBB) "The University of Lahore".

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