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Solubility properties of barley flour, protein isolates and hydrolysates

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Abstract

Protein solubility properties of barley flours (BF), barley protein isolates (BPI) and barley protein hydrolysates (BPH) were determined as a function of pH and NaCI concentration. BPIs were produced from both hulled (BPI-1 and BPI-3) and hull-less (BPI-2 and BPI-4) barley flours. Sodium metabisulphite (BPI-1 and BPI-2) or L-cysteine (BPI-3 and BPI-4) were included in the extraction procedure. BPI-4 was hydrolyzed with Alcalase in order to produce hydrolysates of 3% (BPH-1) and 6% (BPH-2) degree of hydrolysis. Electrophoretic properties of BFs, BPIs and BPHs were examined by SDS-PAGE. The results showed that solubility properties were affected by the changes of pH and ionic strength of the medium in all samples. The solubility properties of barley proteins were especially higher in the strong acidic and basic pH regions. Solubilities of BPI-1 and BPI-2 in distilled water were lower than those of BPI-3 and BPI-4. The lowest solubility was observed around the isoelectric points of BFs and BPIs. SDS-PAGE provided significant information about the monitoring of limited protein hydrolysis that produced large quantities of low molecular weight barley protein fragments with the Alcalase treatment. The solubility properties of BPHs around the isoelectric point were increased as a result of the limited hydrolysis.

Keywords: Barley protein isolate; Hydrolysate; Hull-less barley; Protein solubility

1. Introduction

Barley (*Hordeum vulgare* L.) is an extensively grown cereal and is used mainly for the brewing industry and for animal and poultry feeding. In recent years, increased incorporation of barley into the human diet is recommended, since it is unique among cereals, containing high concentrations of β-glucan, which is known to have cholesterol-lowering effects (Newman, Lewis, Newman, Boik, & Ramage, 1989), regulating blood glucose level and insulin response in diabetics (Cavallero, Empilli, Brighenti, & Stanca, 2002). Although barley is available in both hulled and hull-less forms, hull-less barley, which does not require dehulling, offers some advantages for food uses. Bhatty (1986) reported that hull-less barley can be directly milled to obtain a meal or can be pearled and ground. There is a growing research interest in starch, β-glucan or protein-rich fractions of barley. Although proteins are minor components (8–15%) compared to carbohydrates, their amounts and composition affect industrial uses of barley grain. Major proteins in barley endosperm are the hordeins which are alcohol-soluble proteins (Shewry, 1993). Barley protein is one of the by-products of the barley starch production process (Andersson, Andersson, & Aman, 2001). To find new applications of barley proteins in the food industries, functional properties need to be investigated in detail.

Proteins, as isolates or concentrates, display critical functional properties, such as solubility, emulsifying and foaming effects. Among the functional properties of proteins, solubility is of the most importance, because of its significant influence on the other functional properties (Kinsella, 1976; Vojdani, 1996). It is usually the first func-

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tional property determined during development and testing of new protein ingredients (Zayas, 1997). Many researchers have reported an improvement in some functional properties of proteins using partial enzyme hydrolysis (Casella & Whitaker, 1990; Kinsella, 1976; Zayas, 1997). This is one of the methods most widely used. Hydrolysis is carried out by the action of selected proteolytic enzymes to break specific peptide bonds in a protein. The peptides produced by proteolysis have smaller molecular size and less secondary structure than have native protein and may be expected to have increased solubility near the isoelectric point (Casella & Whitaker, 1990).

There are some differences between plant protein concentrates and isolates in terms of production methods, such as isoelectric precipitation, alcohol precipitation and hot water extraction (Yu, Ahmedna, & Goktepe, 2007). Generally, protein contents of isolates are higher than those of concentrates (Kolar, Richert, Decker, Steinke, & Vander Zanden, 1985; Paredes-Lopez, 1991). An alkaline extraction procedure was developed to produce barley protein concentrate (Wu, Sexson, & Sanderson, 1979). Although there is some information available on the functional properties, such as solubility and emulsifying properties, of barley protein concentrate (Bilgi & Çelik, 2004; Wu et al., 1979), there is not enough information related to the functional properties of barley protein isolates and hydrolysates. In the present study, barley protein isolates (BPI) were prepared from hulled and hull-less barley flours with an ethyl alcohol extraction procedure in the presence of L-cysteine or sodium metabisulphite as reducing agents. Hull-less barley protein isolate was subjected to partial enzyme hydrolysis in order to obtain barley protein hydrolysates (BPH). The flours, BPIs and BPHs were examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Protein solubility properties of barley flour, BPIs and BPHs were investigated at different pH values and ionic strengths.

2. Materials and methods

2.1. Materials

Hulled barley (cvs. Bülbül) and hull-less barley (advanced line, C-738) samples used in this study were obtained from the Field Crops Improvement Center, Ankara, Turkey. The hulled and hull-less barley samples were cleaned on a Carter Dockage Tester, tempered to 14.5% moisture content and milled into straight-grade flour in a Bühler (Germany) laboratory mill with the flour yields of 56.3% and 65.2%, respectively. Protein contents of barley flours were determined by using AACC Standard Method No. 46-12 and converted to protein content (N x 6.25). Moisture contents of flour samples were determined according to AACC Standard Method No. 44-01 (American Association of Cereal Chemists, 1990).

2.2. Preparation of barley protein isolates

Barley protein isolates (BPI) were produced from both hulled (BPI-1 and BPI-3) and hull-less (BPI-2 and BPI-4) barley flours according to the method of Ewart (1980) and Heisel et al. (1986) with slight modifications. Barley flours were extracted with 70% (v/v) ethyl alcohol at a solvent to flour ratio of 3:1. Sodium metabisulphite (SMB) was included in BPI-1 and BPI-2 extractions and L-cysteine in BPI-3 and BPI-4 extractions, at a concentration of 1% (w/v), as a food grade reducing agent in order to increase the protein yield. Extraction was at 20 °C for 2 h with constant mixing on the magnetic stirrer. The extracts were then centrifuged at 20 °C at 900*g* for 30 min. The supernatants were saved and hordeins were precipitated from extract supernatants by the addition of an equal volume of ice-cold 1 M NaCI. After 20 min in an ice bath, the precipitated hordein was recovered by centrifugation (5000*g*, 15 min, 20 °C) and rinsed three times with ice-cold deionized water. Lyophilized hordein isolate was designated as BPI and stored at 4 °C before testing the solubility properties.

2.3. Preparation of barley protein hydrolysates

Hydrolysates were prepared from BPI-4 by a batch process. Enzyme hydrolysis of BPI-4 was carried out according to the method of Adler-Nissen (1986). The pH-stat technique was applied by using the Mettler–Toledo DL21 (NJ, USA) automatic titrator in order to produce the hydrolysates at the desired degree of hydrolysis (DH, %). DH, i.e. the percentage of cleaved peptidic bonds, was calculated by using volume and normality value of the NaOH used to maintain the pH constant (Adler-Nissen, 1986). A commercial end-protease, Alcalase^{TM} or Subtilisin Carlsberg, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). BPI-4 dispersion was prepared in deionized water at 8.3% (w/v) protein concentration. The optimum hydrolysis conditions used were pH 8 and temperature 37 °C. The ratio of enzyme (E) to substrate (S) was 1:300 (w/w). A 60 ml capacity batch system, with constant agitation and controlled temperature, was used. Before starting the hydrolysis, pH of the substrate was adjusted to a value of 8.0–8.2. The appropriate amount of enzyme was dissolved in less than 1 ml of deionized water and added to the slurry incubated at 37 °C. The pH was kept constant at pH 8 by adding 0.1 N NaOH. The hydrolysis reaction was terminated by heating in a boiling water bath for 5 min, when the desired DH was achieved. Then, the reaction mixture was cooled with cold water. Barley protein hydrolysates (BPH) at 3% (BPH-1) and 6% (BPH-2) degree of hydrolysis were freeze-dried, ground and stored at 4 °C until analyzed.

2.4. Determination of solubility properties of barley samples

Protein solubility properties of barley samples were investigated according to the method of Casella and Whi-

taker (1990) with slight modifications. Barley flour and BPI suspensions were prepared in distilled water. Barley flour, BPI-3 and BPI-4 suspensions were also prepared in different concentrations (0.01–0.5 M) of NaCI solutions. BPH suspensions were prepared in distilled water and 0.05–0.1 M NaCI solutions. Solubilities of all suspensions were tested at 0.5% (w/v) concentration. The pHs of suspensions were adjusted to 2–11 by using 0.5 M HCl and 0.5 M NaOH, and stirred at room temperature for 1 h on a magnetic stirrer. Then they were centrifuged at 1640*g* for 15 min at room temperature and the supernatant was filtered through a Whatman No. 1 filter paper to obtain a clear filtrate. The amount of soluble protein in the filtrate was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951) with bovine serum albumin as the standard. The solubility was expressed as a percentage of total protein concentration.

All experiments were performed as duplicate determinations and the mean values and corresponding standard deviations were reported.

2.5. SDS-PAGE method

SDS-PAGE was performed according to the method of Ng and Bushuk (1987) as modified by Fu and Sapirstein (1996). In order to get equal concentrations of proteins, different quantities of flour or lyophilized protein fractions were weighed (based on their protein contents) and dissolved in 1.0 ml of buffer solution (pH 6.8) containing 0.063 M Tris–HCl, 2% (w/v) SDS, 7% (v/v) 2-mercaptoethanol, 20% (w/v) glycerol and 0.01% (w/v) Pyronin Y. Dissolved proteins were heated in boiling water for 3 min and then applied (12.5 μl) to the SDS-PAGE which was carried out in a cooled slab gel unit (Hoefer Scientific Instruments, San Fernando, CA, USA). The gels were stained overnight with Coomassie Brillant Blue G-250 according to Ng and Bushuk (1987). Apparent molecular weights were estimated using wide range molecular weight markers (Sigma, St. Louis, MO, USA).

3. Results and discussion

3.1. Barley samples

Protein contents of hulled and hull-less barley flours were determined as 8.9% and 13.9%, whereas their moisture contents were 9.9% and 11.2%, respectively. Protein contents of BPI-1, BPI-2, BPI-3 and BPI-4 were found to be 86.4%, 86.0%, 83.0% and 85.2% on dry weight basis, while their moisture contents were 4.8%, 3.4%, 5.0% and 3.9%, respectively.

3.2. SDS-PAGE patterns of barley flours and barley protein isolates and hydrolysates

SDS-PAGE patterns of hulled and hull-less barley flours, protein isolates of two barley cultivars and the hydrolysates of BPI-4 obtained from hull-less barley flour are presented in Fig. 1. In this electrophoregram, proteins were termed as by Shewry and Tatham (1990) and Shewry et al. (1994). There were some differences between the relative band intensities and mobilities of the two barley flour samples (Fig. 1, Lanes 1 and 4) as expected from their genotypic differences. The subunit differences in the C hordein and γ and B hordein regions were more pronounced. SDS-PAGE patterns of protein isolates produced by using SMB and L-cysteine were found to be similar to their flour samples in the regions of C hordeins and γ and B hordeins. However, those protein bands were more intense than those of the protein bands of flours. Relative band intensities in the D hordein, albumin and globulin regions decreased distinctly in isolates compared to those of the barley flours. This was more obvious in the protein isolates produced with SMB (Fig. 1, Lane 2 and 5). There were considerable differences between the relative band intensities and mobilities of hydrolysates (Fig. 1, Lanes 7 and 8) compared to that of BPI-4 (Fig. 1, Lane 6). Hydrolysates in the D hordein region were hydrolyzed and disappeared. During hydrolysis, hordeins were degraded by Alcalase, leading to formation of large quantities of low molecular weight (MW) protein fragments. BPH-1 displayed more protein bands in the regions of C hordeins, γ and B hordeins and albumins and globulins than did BPH-2 (Fig. 1, Lanes 7 and 8). In the hydrolysates, the protein bands with the MW of 6500 were more intense than those of the protein isolate and flour sample, due to increased concentration of the low MW peptides.

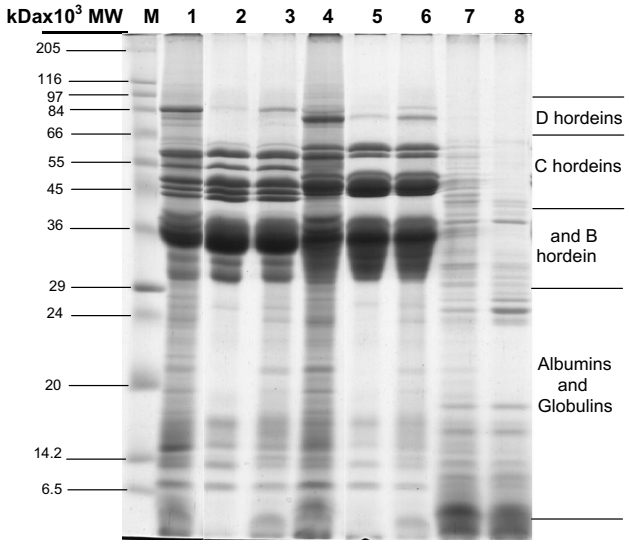

Fig. 1. SDS-PAGE band patterns of hulled and hull-less barley flours, barley protein isolates and hydrolysates. M: wide-range protein markers, 1: hulled barley flour, 2: BPI-1, 3: BPI-3, 4: hull-less barley flour, 5: BPI-2, 6: BPI-4, 7: BPH-1, 8: BPH-2.

3.3. Solubility properties

Effects of the pH on the protein solubility profile of hull-less barley flour in distilled water and various salt concen-

trations are presented in Fig. 2. Protein solubility values were generally higher in distilled water at pH 10 and 11 than at other pH values. Minimum solubility in distilled water was observed around pH 4. However, the protein solubility values at pH 4 and pH 6 were close to each other. Wu et al. (1979) reported that the isoelectric point of barley proteins is close to pH 6. For the barley flour, protein solubility at acidic pH values decreased towards pH 4 and 6 and then increased for basic pH values. This behaviour is similar to those of many other plant flours reported earlier (Adebowale & Lawal, 2004; Chau & Cheung, 1998; McWatters & Cherry, 1977; McWatters & Holmes, 1979; Narayana & Narasinga Rao, 1982). Comparable results were also obtained for hulled barley flour (data not presented).

Our results, concerning the effects of NaCl concentration and pH on the protein solubility of hull-less barley flour solutions, suggested that the presence of NaCl decreased the protein solubility of hull-less barley flour considerably, except for the lowest NaCI concentration (Fig. 2). The protein solubility values in 0.01 M NaCI solution were generally similar to those in distilled water. The protein solubility values in different salt solutions were generally higher at pH 10 and 11. The protein solubility values at pH 4 and pH 6 were close to each other. The results of the present study indicated that the protein solubility of the hull-less barley flour was generally adversely affected by increasing ionic strength. The decreasing effect of NaCl on protein solubility was more evident at acidic pH values than at basic pH values. Similar results have also been reported by other researchers, especially at higher ionic strengths (McWatters & Holmes, 1979; Padilla, Alvarez, & Alfaro, 1996). Comparable results were also obtained for hulled barley flour solutions of cv. Bülbül at all salt concentrations (data not presented). The changes of pH and ionic strength of the medium affected the protein solubility behaviour of both barley flours.

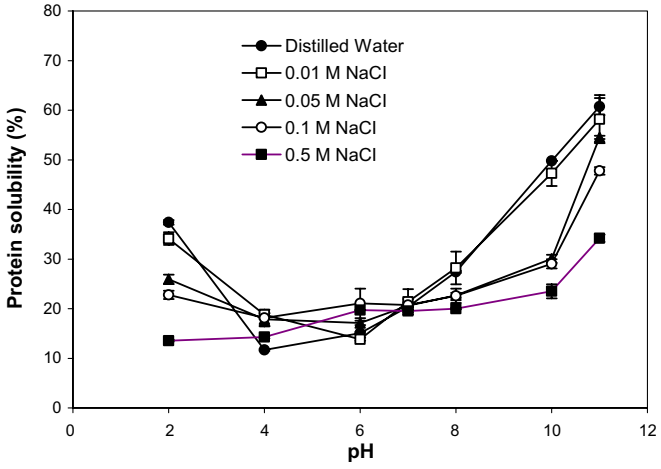

Fig. 2. Effect of pH on the solubility profile of hull-less-barley flour of 0.5% (w/v) in distilled water and different salt (NaCI) concentrations. Bars show standard deviation.

Effects of the pH on the protein solubility profiles of barley protein isolates (BPI-1, BPI-2, BPI-3 and BPI-4) in distilled water are presented in Fig. 3. The isolate of BPI-2 had the lowest protein solubility properties at all pH values. The changes of pH of the medium affected the solubility behaviours of the isolates. The solubility values of the BPI-3 and BPI-4 were especially very close to each other in the strong acidic and basic pH regions. Generally the highest solubility values were detected in the strong basic pH regions (compared to other pH values for all protein isolates). The lowest solubility was observed at pH 6 (around the isoelectric point) for all barley protein isolates. Several researchers have shown that most plant proteins have the lowest protein solubility at their isoelectric point (Ahmedna, Prinyawiwatkul, & Rao, 1999; Bera & Mukherjee, 1989; Bilgi & Çelik, 2004; Franzen & Kinsella, 1976; Sathe, Deshpande, & Salunkhe, 1982; Wu et al., 1979). For all barley protein isolate samples, solubility at acidic pH values decreased towards the isoelectric point and then increased for basic pH values; this behaviour is similar to that of many other plant proteins reported earlier (Ahmedna et al., 1999; Bilgi & Çelik, 2004; Franzen & Kinsella, 1976; Ma & Harwalkar, 1984; Mwasaru, Muhammad, Bakar, & Che Man, 2000; Nielsen, Inglett, Wall, & Donaldson, 1973; Sathe et al., 1982; Wang & Kinsella, 1976). According to the solubility results of all isolates, generally, the protein solubility values of the hulled barley protein isolate (BPI-3) and the hull-less barley protein isolate (BPI-4) both produced by using L-cysteine were higher, at all pH values, than those of the hulled barley protein isolate (BPI-1) and the hull-less barley protein isolate (BPI-2), both produced by using SMB.

Effects of pH on the protein solubility profile of BPI-4 in distilled water and various NaCl concentrations are presented in Fig. 4. Higher solubility values of BPI-4 were

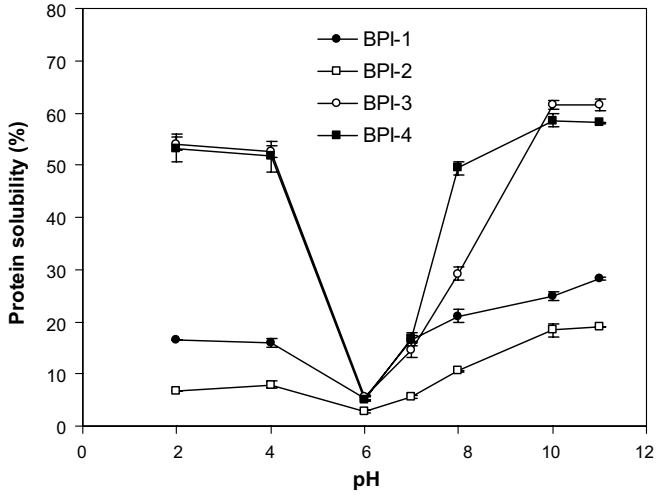

Fig. 3. Effect of pH on the solubility profile of barley protein isolates (BPI), produced from hulled (BPI-1 and BPI-3) and hull-less (BPI-2 and BPI-4) barley flours, at 0.5% (w/v) isolate concentration in distilled water. Bars show standard deviation.

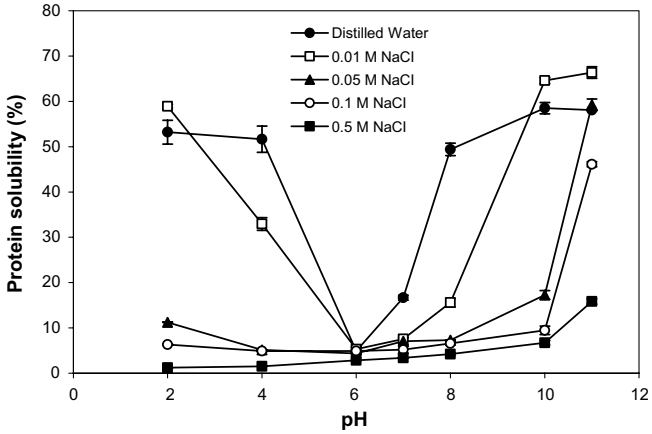

Fig. 4. Effect of pH on the solubility profile of barley protein isolate (BPI-4), produced from hull-less barley flour, at 0.5% (w/v) isolate concentration in distilled water and different salt (NaCI) concentrations. Bars show standard deviation.

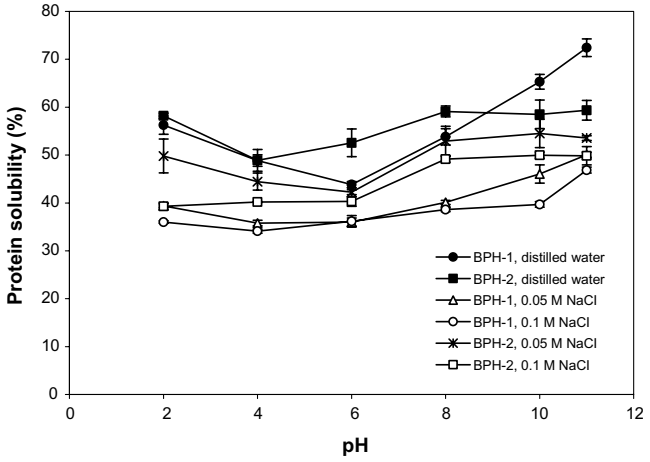

Fig. 5. Effect of pH on the solubility profile of barley protein hydrolysates (BPH-1 and BPH-2), produced from hull-less barley protein isolate (BPI-4), at 0.5% (w/v) concentration in distilled water and different salt (NaCI) concentrations. Bars show standard deviation.

observed in 0.01 M NaCI solutions at pH 11, pH 10 and pH 2 than in distilled water. At other pH values, the protein solubility values of BPI-4 were higher in distilled water than those of salt solutions. Increasing salt concentrations significantly decreased the solubility of isolates, especially in acidic and neutral regions. The isolate suspensions in 0.5 M NaCI exhibited the lowest solubility. Comparable results were also obtained for BPI-3 dissolved in distilled water and salt solutions at the same concentration (data not presented). Generally, the protein solubility of the isolates in salt solutions decreased compared to the solubility in distilled water; this reduction was greater in the acidic pH region than in the basic pH region. This can be explained on the basis of protein–protein and protein–solvent interactions. It was reported that, at low pH, carboxyl groups are protonated and protein has a net positive charge. By addition of NaCl, negatively charged Cl^- ions interact with the positively charged proteins that cause a decrease in electrostatic repulsions and an increase in hydrophobic interactions, leading to a higher tendency for proteins to form insoluble aggregates and a decrease in solubility (Aluko & Yada, 1995; Bilgi & Çelik, 2004; Mwasaru et al., 2000). The results of the present study indicate that the solubility of the protein isolates was generally adversely affected by increasing the ionic strength. Similar results have also been reported by other researchers, especially at higher ionic strengths (Bera & Mukherjee, 1989; Prinyawiwatkul, Beuchat, & McWatters, 1993).

BPI-4 (hull-less barley protein isolate produced by using L-cysteine) was hydrolyzed with Alcalase in order to produce protein hydrolysates at 3% (BPH-1) and 6% (BPH-2) degree of hydrolysis. Effects of pH on the solubility profile of BPH-1 and BPH-2 in distilled water and NaCI solutions are presented in Fig. 5. In distilled water, the highest hydrolysate solubility value was obtained with BPH-1 at pH 11 and the lowest hydrolysate solubility was observed at pH 6, which is close to the isoelectric point of the intact protein. However, a rapid increase of the solubility of the both hydrolysates in distilled water is observed around the isoelectric point (pH 6) compared to BPI-4 (Figs. 4 and 5). Similar results were also reported in other investigations related to food proteins (Chobert et al., 1996; Qi, Hettiarachchy, & Kalapathy, 1997). The apparent increase around the isoelectric point was above 40% for both hydrolysates. The improvement in solubility may be due to the increase in the polar groups during hydrolysis or increase in the reactions between water and hydrophilic groups (Yim & Lee, 2000).

The solubility properties of the hydrolysates in both salt solutions were slightly lower than those of the hydrolysates in distilled water (Fig. 5). Increase in NaCI concentration slightly decreased the solubility of the both hydrolysates. It can also be noted that the solubility properties of both hydrolysates, at all pH levels except for pH 11, considerably increased in salt solutions compared to that of BPI-4 (Figs. 4 and 5). Generally, the solubility values of BPH-2 in salt solutions and distilled water were similar or slightly higher than that of BPH-1. Therefore, the degree of hydrolysis led to some differences in the protein solubility properties of both hydrolysates.

Several researchers have studied the limited hydrolysis by using various proteases in order to increase the solubility properties of the plant proteins (Casella & Whitaker, 1990; Chobert, Sitohy, & Whitaker, 1987; Qi et al., 1997; Were, Hettiarachchy, & Kalapathy, 1997). As a result of the limited hydrolysis, the protein solubility properties of BPHs around the isoelectric point could be increased. Increasing solubility at neutral pH levels might expand the possible use of the BPHs in high protein beverages, emulsion and foam-type foods.

4. Conclusions

According to the protein solubility results of all isolates, the solubility properties of BPI-3 and BPI-4 in distilled water were generally better than those of BPI-1 and BPI-2. Protein solubility properties of barley flour, barley protein isolates and hydrolysates were greatly influenced by changes in pH and ionic strength of the medium. A positive relationship between solubility behaviour of barley flour and isolates was determined as the values in the acidic pH region decreased toward the pH values close to the isoelectric point and increased for the basic pH values in distilled water. Enzymatic hydrolysis of BPI-4 led to marked increase in solubility at neutral pH values, especially around the isoelectric point of the intact protein. The results of the present investigation indicate that the protein solubilities of the barley flour, barley protein isolates and hydrolysates were generally adversely affected by increasing the ionic strength. High protein solubility properties may admit BPI and BPH as useful ingredients for various food applications; however, further studies are needed to investigate the parameters affecting functionality for use in food systems. The barley proteins used in the present study were extracted directly from barley flour. Preparation of barley protein isolates is another possibility for utilization of barley protein, a by-product of the barley starch industry. Therefore, solubility and other functional properties of the protein fractions, obtained from the by-products of the starch industry, should also be investigated in detail.

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