Effect of enzymatic hydrolysis on the functional, antioxidant, and angiotensin I-converting enzyme (ACE) inhibitory properties of whole horse gram flour

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Received: 24 May 2018 / Revised: 9 July 2018 / Accepted: 14 July 2018 / Published online: 23 July 2018

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Abstract Horse gram hydrolysate (HGH) with different degree of hydrolysis (DH) (20, 25, 35, 40, and 45%) was prepared from whole horse gram flour using alcalase. The amino acid composition of HGH showed the presence of essential amino acids. The alcalase hydrolysis (DH $\geq 20\%$) increased protein solubility with a notable difference in the pH range of 3–5 ($p < 0.05$). The emulsifying activity and stability of HGH improved with increase in pH, especially at DH $\geq 25\%$ ($p < 0.05$). With increase in DH, the foaming properties reduced while the antioxidant and angiotensin I-converting enzyme inhibitory activities increased. Sensory evaluation showed no significant difference ($p > 0.05$) in preference between control soup and soup mixed with HGH. Thus, these results suggest the possibility of HGH to be used as an appropriate functional ingredient with different food applications including in management of oxidative stress as well as in controlling hypertension.

Keywords Horse gram hydrolysate · Enzymatic hydrolysis · Alcalase · Functional properties · Antioxidant · ACE inhibitory

Introduction

In many developing countries, legumes play an important role in human nutrition as a cheap source of dietary proteins. Horse gram (*Macrotyloma uniflorum*) is an underutilized legume grown extensively in the tropics and subtropical region (Prasad and Singh, 2014). It is a good source of proteins (17.9–25.3%), carbohydrates (51.9–60.1%), dietary fibre, iron, and molybdenum. It has also been recognized by the National Academy of Sciences in 1979 as one of the potential food sources for the future (Sreerama et al., 2008). Hence, this legume has gained interest from researchers and food manufacturers to be used as a functional food ingredient.

Functional properties of food products, conferred mainly by protein component present in food, influence its use in the final food product (Wouters et al., 2016). Processing of horse gram legume or its flour by milling, germination, popping, soaking, and cooking has shown improvement in the functional and nutritional quality of horse gram in addition to increased utilization of this legume as human food (Ghumman et al., 2016; Sasikala et al., 2011; Sreerama et al., 2008; Sreerama et al., 2010). Enzymatic hydrolysis is another such processing method which is known to improve the functional properties of food proteins by production of peptides of different sizes.

In recent years, many dietary proteins have been reported to possess specific bioactivities including angiotensin I-converting enzyme (ACE) inhibitory, antithrombotic, antioxidative, immunomodulatory, antimicrobial, opioid, and mineral binding activity. These activities are attributed to biologically active peptides that are encrypted within the parent protein and can be released by use of exogenous enzymes, during gastrointestinal digestion, food processing, or fermentation (Bhat et al., 2015). Food

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products containing ACE inhibitory peptides (to decrease blood pressure) are the most widely studied group of bioactive peptides (Hernández-Ledesma et al., 2011). Synthetic drugs such as captopril and enalapril exhibit some side effects and hence, ACE inhibitors from food has been of interest currently even though their potency is less than that of these commercial drugs. Another factor that causes diseases such as cancer, rheumatoid arthritis, and atherosclerosis is the presence of highly reactive free radicals. Hence, there is a growing interest among researchers to obtain natural antioxidants in the form of bioactive peptides from food (Chi et al., 2015).

Protein hydrolysate preparation by a specific enzyme can be controlled to produce hydrolysates with different degree of hydrolysis which in turn can affect the functional properties as well as the generation of bioactive peptides. Most investigators have used either protein isolate or extracted specific protein/s from legumes for the production of protein hydrolysates in their work (Barac et al., 2012; Boschin et al., 2014; Wani et al., 2015). Also, there are no reports on preparation of protein hydrolysates from horse gram seed flour. The functional properties of legume flours are not solely dependent on proteins but also on other food components like carbohydrates possibly through protein–carbohydrate interactions (Kaur and Singh, 2005). In addition to this, it has been reported in the literature that other compounds from foods such as phenolics and oligosaccharides possess *in vitro* antioxidant as well as ACE inhibitory activities (Pihlanto et al., 2008). Therefore, the objective of the present study was to evaluate the functional, antioxidant, and ACE inhibitory potential of protein hydrolysates obtained from whole horse gram flour by enzymatic hydrolysis.

Materials and methods

Materials

Horse gram (HG) seeds and instant tomato soup premix (Farm Tomato Soup, Reliance, India) were purchased from a local market in Mumbai, India. HG seeds were ground to fine powder in a laboratory mill of mesh size 0.8 mm (Perten Instruments, Sweden). Alcalase 2.4L (2.4 AU/g) was obtained from Novo Nordisk (Bagsvaerd, Denmark). 2,4,6-trinitro-benzenesulfonic acid (TNBS), Hip-His-Leu (HHL), 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Chemicals (St. Louis, MO, USA). All other chemicals were of analytical or HPLC grade.

Preparation of HG hydrolysate (HGH)

HG flour was mixed with water in the ratio of 1/10 (w/v) and was adjusted to pH 8 with 0.1 M NaOH. The mixture was incubated at 55 °C and alcalase was added at an enzyme to substrate (E/S) ratio of 1:10 (v/w). Aliquots were taken from this mixture at specific time intervals of 0.5, 1, 2, 3, 4, and 5 h, during which pH was kept constant. After incubation, the enzyme was inactivated at 90 °C for 10 min. A part of this suspension was used for free amino nitrogen, antioxidant and ACE inhibitory studies, while the remaining was freeze-dried to obtain HGH in powder form and was stored at − 20 °C. The degree of hydrolysis, defined as the percentage of peptide bonds cleaved, was estimated by the reaction of free amino groups with TNBS (Adler-Nissen, 1979).

Proximate composition of HGH

Proximate composition of HGH powder with DH of 45% (DH45) was investigated according to the AOAC methods (AOAC, 2000).

Amino acid composition of HGH

Amino acid composition of vacuum dried sample (10 mg of DH45 HGH) was determined after hydrolysis in 6 M HCl at 110 °C for 24 h as described by Li et al. (2005) with some modifications. The o-phthal-dialdehyde (OPA) amino acid derivatives were eluted on Hypersil ODS C18 column (10 µm, 250 × 4.6 mm) (Thermo Scientific, USA) using gradient of 12.5 mM phosphate buffer, pH 7.2 (buffer A) and buffer A containing 50% acetonitrile (buffer B) with flow rate of 1 mL/min. The gradient was performed as follows: 0–5 min solvent A; 5–35 min solvent B, 50%; 35–40 min solvent B, 100%; 40–45 min solvent B, 100%. Detection of derivatized amino acid was performed at 330 nm.

Functional properties of HGH

Solubility

To determine protein solubility, 200 mg of control and hydrolysate samples were suspended in 20 mL of distilled water in nine different tubes and the pH of the solution in individual tube was adjusted to 2, 3, 4, 5, 6, 7, 8, 9, and 10 with 0.1 M HCl or NaOH. The mixture was then centrifuged at 10,000 × g for 10 min. The nitrogen content in the supernatant was determined by Kjeldahl method and protein solubility was expressed in terms of g soluble nitrogen per 100 g nitrogen in sample.

Emulsifying properties

Emulsifying properties of control unhydrolyzed flour and HGH at different pH (3–9) were determined according to the method of Jamdar et al. (2010). The emulsifying activity index (EAI) and emulsion stability (ES) were calculated as follows:

$$\text{EAI}(\text{m}^2/\text{g}) = (2 \times 2.303 \times A_{500 \text{ nm}}) / F \times \text{protein weight (g)}$$

where A is the absorbance of homogenized mixture at 500 nm and F is the oil volume fraction, which is 0.25.

$$\text{ES}(\%) = A_{10}/A_0 \times 100$$

where A_0 and A_{10} are the absorbance values measured at 500 nm, immediately and 10 min after emulsion formation, respectively.

Foaming properties

Foaming capacity (FC) and foaming stability (FS) of control flour and HGH at different pH (3–9) were determined according to the method of Jamdar et al. (2010). The FC was calculated according to the following equation:

$$\text{FC}(\%) = [(A_0 - B)/B] \times 100$$

where A_0 and B is the volume after and before whipping (mL), respectively.

FS was calculated as follows:

$$\text{FS}(\%) = [(A_t - B)/B] \times 100$$

where A_t is the volume after standing (mL) and B is the volume before whipping (mL).

Antioxidant activities of HGH

ABTS radical scavenging activity

The scavenging effect of HGH on ABTS radical was determined by the decolorization assay of Re et al. (1999) with minor modifications. Appropriately diluted hydrolysate extract (50 μL) was allowed to react with 150 μL of ABTS solution and decrease in absorbance was measured spectrophotometrically at 734 nm after 6 min using a microplate reader (Multiskan GO, Thermo Scientific, USA). The results were expressed as Trolox equivalent (μM) per mg of sample.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacity of HGH was estimated according to the procedure described by Benzie and Strain (1996) with some modifications. Freshly prepared FRAP reagent

(150 μL) was mixed with 50 μL of appropriately diluted hydrolysate sample. After 10 min of incubation at room temperature, absorbance was measured at 593 nm in a microplate reader (Multiskan GO, Thermo Scientific, USA). FRAP activity was expressed as Ferrous equivalent (μM) per mg of sample.

Evaluation of total antioxidant capacity (TAC)

The total antioxidant capacity of HGH was evaluated by the method of Prieto et al. (1999) with slight modifications. An aliquot of 0.1 mL of HGH solution was combined with 0.4 mL of TAC reagent. The solution was mixed well and incubated at 90 $^{\circ}\text{C}$ for 90 min. The absorbance was analysed spectrophotometrically at 695 nm. The TAC values were expressed as α -tocopherol ($\mu\text{mol}/\text{mL}$) equivalent per mg sample.

Metal chelating activity

The Fe^{2+} chelating activity of the extract was determined using the method of Decker and Welch (1990). Diluted HGH sample (100 μL) was mixed with 10 μL of 2 mM $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 180 μL of acetate buffer (pH 5.9) and 10 μL of 5 mM ferrozine. This mixture was reacted for 10 min at room temperature and absorbance was measured at 562 nm. The chelating activity was expressed as μM of EDTA equivalent per mg sample.

ACE inhibitory activity of HGH

ACE inhibitory activity was determined by the method followed by Jamdar et al. (2010) with some modifications. In the assay, 20 μL of HGH extract (2 mg sample with 5.71 mg/mL final concentration) was incubated with 15 μL of ACE extracted from pig lungs (Jamdar et al., 2010) for 10 min at ambient temperature; further incubated with 250 μL of 4 mM HHL (prepared in 50 mM HEPES buffer containing 0.3 M NaCl, pH 8.3) at 37 $^{\circ}\text{C}$ for 30 min. The reaction was terminated with 200 μL of 1 M HCl. The resulting hippuric acid was extracted with 1 mL of ethyl acetate. After centrifugation ($10,000 \times g$ for 10 min), 700 μL of the upper layer was transferred into another tube and evaporated. The hippuric acid was dissolved in 1 mL of distilled water and absorbance was measured at 228 nm using UV spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan). Distilled water was used instead of sample solution for the control experiment. ACE inhibition (%) was calculated as follows:

$$\% \text{ ACE inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Sensory evaluation of hydrolysate (DH40) incorporated soup

Instant soup was prepared according to the procedure given on the pack. Hydrolysate (DH40) with highest ACE inhibitory as well as with antioxidant activity was added to the soup (25% w/w). For sensory evaluation, triangle test was performed to determine the sample differences. Three coded samples were provided to 20 untrained sensory panelists and were asked to choose the odd sample. The samples were presented as AAB, ABA, BBA and BAB where 'A' indicated the control soup and 'B' indicated the hydrolysate incorporated soup. Each combination was provided for the same number of times. Also, preference test was conducted wherein the panelists were asked to decide if they preferred the control or the hydrolysate incorporated soup. The statistical analysis was performed by analysing the number of correct identification to show significance (Roessler et al., 1978).

Statistical analysis

All the experiments were conducted in triplicates and values were expressed as mean $\pm$ standard deviation. Data were analysed by one-way ANOVA (Analysis of Variance) and the mean comparison was performed by Duncan's multiple range test using IBM, SPSS Statistics software v.20.0 (IBM SPSS Inc., Chicago, IL, USA). All tests were considered to be statistically significant at $p < 0.05$.

Results and discussion

Effect of hydrolysis on horse gram powder, proximate composition and amino acid analysis of HGH

The extent of hydrolysis of horse gram protein after 0.5, 1, 2, 3, 4, and 5 h by alcalase, measured as degree of hydrolysis, was 20% ($18.25 \pm 1.66\%$), 25% ($25.47 \pm 0.54\%$), 35% ($33.71 \pm 0.0\%$), 40% ($40.30 \pm 0.36\%$), 44% ($43.47 \pm 0.54\%$), and 45% ($44.35 \pm 1.79\%$), respectively. As shown in Fig. 1, the hydrolysis proceeded rapidly during initial phase of hydrolysis (up to 1 h) which decreased subsequently and reached a steady state phase after 4–5 h of incubation exhibiting DH of 45% (DH45). This typical hydrolysis curve was in agreement with Chalamaiah et al. (2010) and Ghribi et al. (2015a). The increase in DH indicated that alcalase enzyme, which has a broad specificity for protein hydrolysis, cleaved the proteins of HG into smaller peptides by the end of 5 h.

Fig. 1 Changes in degree of hydrolysis (DH) of whole horse gram flour treated with alcalase at different incubation time. The values represent mean of three independent experiments $\pm$ S.D

Table 1 (A) Proximate composition and (B) amino acid composition (mg/g) of horse gram hydrolysate (DH45)

Parameters	
(A) Proximate composition (%)	
Protein	21.18 $\pm$ 0.8
Moisture	12.11 $\pm$ 0.11
Ash	2.57 $\pm$ 0.24
Fat	0.74 $\pm$ 0.15
Carbohydrate	63.09 $\pm$ 1.24
(B) Amino acid composition (mg/g of hydrolysate)	
Aspartic acid ^a	20.7 $\pm$ 0.9
Glutamic acid ^a	28.7 $\pm$ 0.8
Arginine	12.8 $\pm$ 0.4
Serine	10.5 $\pm$ 0.8
Tyrosine	5.3 $\pm$ 0.2
Lysine	28.3 $\pm$ 2.5
Leucine	26.6 $\pm$ 1.2
Isoleucine	15.6 $\pm$ 0.1
Alanine	13.3 $\pm$ 0.9
Phenylalanine	9.3 $\pm$ 1.2
Threonine	7.4 $\pm$ 0.8
Methionine	11.4 $\pm$ 0.9
Valine	6.4 $\pm$ 0.5

Values represent mean of three independent experiments $\pm$ S.D

^aValues for glutamic acid/glutamine and aspartic acid/asparagine are reported cumulatively since asparagine and glutamine are partially converted to aspartic acid and glutamic acid, respectively during hydrolysis

The proximate composition of HGH (DH45) is presented in Table 1A. The crude protein content of HGH was 21.18% and was similar to those reported by Moktan and Ojha (2016) in raw HG flour (20.90%). Thus, alcalase hydrolysis of HG flour did not cause any significant change in the protein content. The amino acid composition of HGH (DH45) is presented in Table 1B. It shows that the hydrolysate is a rich source of glutamic acid and glutamine (28.7 mg/g), lysine (28.3 mg/g), leucine (26.6 mg/g), aspartic acid and asparagine (20.7 mg/g) while the content of methionine (11.4 mg/g) is less. However, the tryptophan and cysteine content could not be determined in the present study, maybe because of the sensitivity of the amino acid to acid hydrolysis. Moreover, since derivatization of imino acids by OPA is not possible, the proline content also could not be reported. This amino acid composition of HGH is comparable to that reported for raw HG by Prasad and Singh (2014). Thus, it suggests that conditions of hydrolysis did not affect the amino acid composition of HG, especially the essential amino acids.

Functional properties of HGH

Solubility

Solubility is one of the most important functional properties of protein hydrolysates as it affects other properties such as emulsification and foaming (Wouters et al., 2016). The percent nitrogen solubility of control and HGH at different pH values are shown in Fig. 2.

The control showed lowest solubility (21–47%) in the pH range of 3–6 which is in agreement with the study performed by Chalamaiah et al. (2010) and this low solubility can be attributed to the isoelectric point of HG

Fig. 2 Effect of degree of hydrolysis on nitrogen solubility (%) of whole horse gram flour hydrolyzed using alcalase at different pH values. The values represent mean of three independent experiments $\pm$ S.D

proteins. However, hydrolysis of the protein improved their solubility, particularly in the pH range 3–6. Furthermore, as reported by Chalamaiah et al. (2010) and Ghribi et al. (2015b), the solubility increased with increase in DH. Overall, the nitrogen solubility after hydrolysis of HG seed flour with alcalase could be improved to as high as 55–70% at all the pH values under study. A maximum solubility of 70% was achieved at DH of 45% in the pH range of 8–10. The improved solubility of hydrolysates may be due to generation of small molecular size peptides and exposure of charged and polar groups that increased hydrophilicity and thus, enhanced the interaction with the surrounding aqueous phase (Chalamaiah et al., 2010; Wani et al., 2015).

Emulsifying properties

It is known that the emulsifying property of a protein or peptide is attributed to their adsorption at the interface to form a protective film around oil droplets during homogenization (Wasswa et al., 2007; Wouters et al., 2016). The values of EAI and ES of control and HGH with varying DH obtained in the pH range of 3–9 are presented in Fig. 3A and B, respectively. EAI of control and HGH were lowest in the acidic pH (3–5) since it is close to the isoelectric point and maximum was at pH 8 and 9 ($p < 0.05$). HGH with DH of 20, 25 and 35% showed higher EAI at pH 3 and 4 when compared with DH of 40 or 45%. There was an increase in EAI of all the hydrolysates at the alkaline pH range of 5–9 since change in pH has been shown to affect the surface charge of protein or peptide (Barac et al., 2012). The hydrolysates with DH of 25 and 35% exhibited the lowest EAI at pH range of 5–9 than that of other HGH. Moreover, the EAI of hydrolysate DH40 and DH45 in the alkaline pH range was higher when compared to hydrolysates with lower DH values. Emulsion stability of control was poorer than that of the hydrolysates at all pH values taken for study ($p < 0.05$), especially at pH 4 and 5. ES in the acidic range of pH 3–5 at various DH of HGH was lower than at pH 6–9 wherein it was observed that the stability of HGH at pH 6–9 remained similar and was independent of the DH (Guan et al., 2007; Jamdar et al., 2010). Difference in peptide chain length and molecular structure of protein (molecular weight and hydrophobicity) may account for this change in emulsifying ability of hydrolysates (Wouters et al., 2016).

Foaming properties

Figure 3C and D shows the effect of pH on the FC and FS of control powder and HGH. As shown in the Fig. 3C, HGH displayed lower FC than the control ($p < 0.05$). Foam stability of control was poor at pH 3 and 4 and was found to increase from pH 5–9 (Fig. 3D). But, FS of HGH

Fig. 3 Emulsifying and foaming properties of horse gram hydrolysates as influenced by pH (A) emulsifying activity index (EAI), (B) emulsion stability (ES), (C) foaming capacity (FC), and

(D) foaming stability (FS). The values represent mean of three independent experiments $\pm$ S.D. Values with different superscript letters are significantly different ($p < 0.05$)

was very poor and it decreased with increase in DH. At pH 5 and 6, hydrolysate solution with DH of 40 and 45% shows FS which is near to zero ($p < 0.05$). Jamdar et al. (2010) and Liu et al. (2010) reported poor foaming properties of peanut protein hydrolysate and porcine plasma protein hydrolysate, respectively, as DH increased. This decrease in foaming ability of HGH with increase in DH could be due to the fact that low molecular weight peptides do not have the strength to provide stable foams as well as its inability to maintain a well-ordered orientation at the

interface which is required for foaming (Wouters et al., 2016). In addition, hydrophobicity of peptides after hydrolysis has also been shown to correlate with foaming characteristics.

Antioxidant activities of HGH

ABTS radical scavenging activity of HGH

ABTS radical scavenging assay can be applied to both lipophilic and hydrophilic compounds (Corrêa et al., 2011). As shown in Fig. 4A, there was increase in scavenging of ABTS radicals as expressed by the Trolox equivalents, as DH increased. The control and hydrolysate with DH of 20 and 25% showed lowest radical scavenging activity of 188.6, 179.8 and 195 μM Trolox equivalents/mg, respectively. However, a drastic increase in activity was observed in hydrolysate with DH of 35% (273.2 μM Trolox equivalent/mg) ($p < 0.05$). The highest activity in HGH was demonstrated by DH40 (304.2 μM Trolox equivalent/mg) and DH 45 (316.2 μM Trolox equivalent/mg). During enzymatic treatment, the release of peptides from intact protein might have led to increase in scavenging activity

which has also been reported by Pihlanto et al. (2008) in potato hydrolysates.

FRAP assay for HGH

The FRAP assay measures the reducing ability of compounds present in a given medium. The antioxidant potential of control unhydrolyzed and hydrolyzed horse gram extracts was estimated from their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II) complex. As DH increased, there was significant increase ($p < 0.05$) in reducing ability of hydrolysates (highest being 1104.5 μM Ferrous equivalent/mg of DH45) than the unhydrolyzed extract (870.5 μM Ferrous equivalent/mg) [Fig. 4B].

Total antioxidant capacity (TAC) of HGH

The TAC of control and HGH extract is depicted in Fig. 4C. All the hydrolysates showed similar TAC values

Fig. 4 Antioxidant activities of horse gram hydrolysate extracts (A) radical scavenging activity by ABTS assay, (B) ferric reducing antioxidant power, (C) total antioxidant capacity, and (D) metal chelating activity. The values represent mean of three independent experiments $\pm$ S.D. Values with different superscript letters are significantly different ($p < 0.05$)

(30–35 $\mu\text{mol/mL}$ Tocopherol equivalent/mg sample) as that of control extract (33.2 $\mu\text{mol/mL}$ Tocopherol equivalent/mg sample) ($p > 0.05$). The results indicate that hydrolysis of HG proteins into peptides was not able to contribute any change towards TAC and that the TAC content observed might be due to other components like phenolics present in the extract.

Metal chelating activity of HGH

As shown in Fig. 4D, metal ion (Fe^{2+}) chelating activity of HGH increased slightly till a certain DH (DH of 25 and 35%) ($p < 0.05$) after which there was no significant increase in Fe^{2+} ion chelating activity. Hydrolysis of proteins may have rendered hydrolysates with higher metal chelating activity. Hydrolysates from chickpea (Ghribi et al., 2015a) and porcine plasma (Liu et al., 2010) also showed an increase in Fe^{2+} ion binding activity. Chelating agents reduce the availability of transition metals such as Fe^{2+} , Cu^{2+} , Co^{2+} in foods and retard the oxidation reaction (Liu et al., 2010).

Fig. 5 ACE inhibitory activity of horse gram hydrolysate extracts expressed as percent inhibition. The values represent mean of three independent experiments $\pm$ S.D. Values with different superscript letters are significantly different ($p < 0.05$)

ACE inhibitory activity of HGH

The ACE inhibitory activity of control and its hydrolysate extract expressed as percent ACE inhibition is presented in Fig. 5. The control showed a negligible ACE inhibition of around 4%, which increased to the extent of 77% inhibition in HGH. This highest ACE inhibitory activity was demonstrated by DH40 hydrolysate ($p < 0.05$). There was increase in inhibition capacity as degree of hydrolysis progressed. However, the DH45 hydrolysate exhibited a decrease in ACE inhibition when compared to DH40 hydrolysate. Similar trends have been observed for different protein hydrolysates, suggesting that there might be an optimal degree of hydrolysis, after which the hydrolysis of ACE inhibitory peptides leads to the production of inactive peptides or free amino acids (Corrêa et al., 2011).

Sensory analysis of DH40 incorporated soup

With 20 untrained panelists, a minimum number of 11 correct identifications of odd sample for triangle test and 15 agreeing judgments for preference test were required to establish significance at level of 0.05 (Roessler et al., 1978). As presented in Table 2, the analysis of triangle test showed 15 correct identifications implying that there were significant differences ($p < 0.05$) between control and DH40 hydrolysate incorporated soup. However, in preference test, 10 panelists preferred hydrolysate incorporated soup while the other 10 preferred control soup suggesting that there was no significant difference ($p > 0.05$) with regards to preference of samples (Table 2). Peptides and free amino acids formed during hydrolysis have been known to affect the flavor of food. Due to buffering action of peptide and presence of free amino acids like Glu and Asp, there is a change or improvement in the taste of food by formation of different flavors (basic and umami flavors, respectively) and by its interaction with other flavor components in food (Zhang et al., 2013).

For successful acceptance of legume seed flour in the food system, it is necessary for it to possess

Table 2 Sensory evaluation of soup mix supplemented with horse gram hydrolysate (DH40)

Test	No. of panelists	No. of correct/agreeing judgments	Minimum of correct/agreeing judgments at 5% significance level*	Significance*
Triangle test	20	15 ^a	11	$p < 0.05$
Preference test	20	10 ^b	15	$p > 0.05$

^aNo. of panelist members who perceived the odd sample between control and hydrolysate incorporated soup

^bNo. of panelist members who preferred hydrolysate incorporated soup over control soup

*Roessler et al. (1978) (Control and hydrolysate incorporated soup are significantly different if $p < 0.05$)

suitable functional characteristics. The results demonstrate that the hydrolysate prepared from whole HG flour, particularly HGH with DH of 40%, improved the functional properties along with its bioactivity (antioxidant and ACE inhibitory activities). Furthermore, sensory analysis showed acceptance of hydrolysate incorporated food by the panelists. This suggests the effective incorporation of HGH as a functional food ingredient with enhanced health benefits. Moreover, since whole horse gram flour was used in this study instead of protein isolate, there was no waste generated during HGH production.

Acknowledgements Authors would like to thank Department of Biotechnology (DBT), India for funding this research (DBT-JRF/2011-12/205).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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