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Evaluation of quality parameters and antioxidant properties of protein concentrates and hydrolysates of hyacinth bean (*Lablab purpureus*)

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Abstract

The hyacinth bean protein hydrolysates (HPHs) were derived from hyacinth bean protein concentrate (HPC) by enzymatic hydrolysis following isoelectric method using pepsin enzyme. The HPH displayed strong antioxidant activities than hyacinth flour and HPC, where released amino acids were the major contributors to enhanced antioxidant activities. The IC_{50} values for HPH and ascorbic acid (AA) were 0.052 and 0.020 mg/ml correspondingly. The total phenolic content (TPC) of HPH and HPC were 35.1 and 22.03 mg GAE/g, respectively. The antioxidant potential of both HPC and HPH was assessed by adding those components to apple juice at a rate of 3 g/L and kept at ambient temperature for 6 days. Both HPC and HPH delayed the development of oxidizing products during 6-day storage where HPH exhibited significantly higher antioxidant activity to that of HPC. The HPH reduced the oxidizing substances by 79.58%, 65.1%, and 62.03% at Days 0, 1, and 6, respectively, compared with control. These findings demonstrate that the hyacinth hydrolysates offer a good potential for use in the food industry as natural antioxidants.

KEYWORDS
antioxidant activity, DPPH activity, oxidizing substances, protein concentrate, protein hydrolysates

1 | INTRODUCTION

The hyacinth bean (*Lablab purpureus*), which belongs to the Leguminosae (Fabaceae) family, is abundantly grown in the tropics and subtropics. It comprises 18% to 25% protein, 54% to 63% carbohydrates, 1.1% lipid, and significant quantity of energy (Hossain et al., 2016). Essential amino acids like lysine and leucine are also found in the hyacinth bean seeds (Shaahu et al., 2015). It has both nutraceutical and pharmaceutical properties (Morris, 2009). Due to the presence of the excellent combination of amino acids, higher level of protein bioavailability, these seeds can be counted as an appropriate source of usable protein (Subagio, 2006). However, hyacinth beans also possess a few antinutritional factors (ANFs) including lectins, trypsin inhibitor, and phytate (Subagio, 2006). Enzyme inhibitors and lectins both impair protein digestion and nutrient absorption, with lectins having detrimental consequences in the intestine. However, cooking or other processing methods can significantly reduce these negative effects of ANFs (Lajolo & Genovese, 2002; Roy et al., 2021).

A significant concern in the food industry is the oxidation of lipids in food products as it significantly reduces the shelf life of food items. Oxidation may release certain free radicals that are accountable for lipid decomposition, which inevitably creates unpleasant rancid odors, flavors, and harmful reaction products, that is, free radicals

(McClements & Decker, 2000). Such free radicals are also responsible for numerous medical disorders, such as ageing, heart disease, cancer, muscular dystrophy, hypertension, cardiac and myocardial infarctions, and arthritis (Wang et al., 2007). Lipid peroxidation and development of free radicals in food products must be avoided in order to avoid food quality deterioration and to provide a defense mechanism in the human body against multiple diseases (Halliwell et al., 1995). Nonetheless, enzymatic browning or discoloration due to oxidation is an important point of consideration in case of low antioxidant-rich product especially apple juice. The apple juice has low antioxidant activity (IC_{50} value: 2.5 mg/ml) and low total phenolic content (5.85 mg GAE/g) than other common fruit juices (Beh et al., 2012). It also possesses relatively low content of vitamin C (Glevitzky et al., 2008) which declined significantly during heat processing such as pasteurization. Therefore, apple juice is prone to enzymatic browning due to the oxidation of phenols into quinones which is undesirable. It can be prevented by adding antioxidant-rich components. Antioxidants, which prevent the oxidation of carbon-based compounds, are necessary for food processing and the protection of living systems against oxidative stress (Masuda et al., 2003). By blocking the formation or continuation of oxidizing chain reactions, they can slow or stop oxidation due to their ability to scavenge free radicals (Piccolella et al., 2008).

In food industry, several synthetic antioxidants are being used to prevent lipid peroxidation in foods, such as BHT, BHA, and propyl gallate (PG). As they may potentially induce liver failure, mutagenicity, and neurotoxicity, these synthetic antioxidants can be hazardous to human health (Vijayabaskar & Shiyamala, 2012). The synthetic antioxidants are also reported to have carcinogenic effects (Velioglu et al., 1998). However, to ensure food safety, their use in food products is strictly regulated by regulatory agencies such as the U.S. Food and Drug Administration (FDA), World Health Organization (WHO) (Je et al., 2007). Therefore, in recent years, the demand for natural antioxidants is increasing dramatically for the sustainable safety and to prevent harmful health effects of artificial antioxidants (Alam et al., 2020; Sarkar, Ahmed, et al., 2020; Sarkar et al., 2021). This need has prompted interest in discovering additional natural antioxidant substances are growing tremendously worldwide. Natural antioxidants like ascorbic acid (AA), vitamin A, herbal extracts, and tea extracts are widely being utilized as sources of antioxidants in food systems (Sarkar, Rahman, et al., 2020; Shahidi, 2000). Recently, some research has shown that protein hydrolysates from various sources, including plant-based products, have the potentiality to exhibit antioxidant activities against the lipid oxidation (Peña-Ramos et al., 2004). Antioxidant potential has been detected in protein hydrolysates obtained from several sources including whey, soy protein (Peña-Ramos & Xiong, 2003), egg yolk (Sakanaka et al., 2004), prawn (Suetsuna, 2000), tuna cooking juice (Jao & Ko, 2002), yellowfin sole frame (Je et al., 2005), herring (Sathivel et al., 2003), and mackerel (Wu et al., 2003). In addition, food protein hydrolysates also display probable pharmacological functions, for example, antihypertensive, antibiotic, immunomodulatory, anticarcinogenic, antibacterial, and lipid-lowering actions (Korhonen & Pihlanto, 2006; Meisel, 2004).

Hyacinth bean seed is a potential source of healthy food for human consumption due to their low fat, nutraceutical, and pharmaceutical properties (Hossain et al., 2021; Morris, 2009). The main objectives of the study were to evaluate the quality parameters, antioxidant activities of protein concentrates (hyacinth bean protein concentrate [HPC]) and hydrolysates (hyacinth bean protein hydrolysate [HPH]) of hyacinth bean, and the effects of those components in a model food (apple juice).

2 | MATERIALS AND METHODS

2.1 | Raw materials

Hyacinth beans were purchased from local market of Sylhet, Bangladesh, and sorted for uniform size and shape. Beans were milled into flour and forced through a mesh (0.2 mm), and flour were packaged and stored at 4°C in a sealed container for further analysis and use.

2.2 | Protein isolation

Proteins from hyacinth beans were isolated according to the method of Roy et al. (2020) with subtle modification. In distilled water, flour was distributed at a ratio of 1:5. The mixture was calibrated using a digital pH meter to pH 9.5 agitating for 1 h. Then, the slurry was centrifuged at 8000 rpm for 30 min, and the precipitates were removed. The obtained filtrate was adjusted to pH 4.5 and centrifuged as above. Finally, the collected precipitates were freeze-dried after washing and stored in sealed polyethylene bags. All the experiments and subsequent analysis/evaluations were done using three replicates.

2.3 | Proximate chemical composition analysis

The protein content (*N*) was estimated in compliance with the official methods of AOAC (1995) by Kjeldahl method. Subsequently, the crude protein content was determined by multiplying the *N* with 6.25 ($N \times 6.25$) (Hossain et al., 2016). Both moisture and ash content were evaluated by following the standard methods of AACC (2000). Total fat was calculated by the method of Roy et al. (2020). Total carbohydrate content was determined by method of Rui et al. (2011).

2.4 | Preparation of hyacinth protein hydrolysates

The HPH was produced using enzymatic protein hydrolysis induced by pepsin enzyme (Carrasco-Castilla et al., 2012; Rui et al., 2013). Protein suspension was provided in a sodium acetate buffer for pepsin treatment and the value of pH was tuned to 3.6. Throughout the entire process, the temperature was maintained at 37°C, and the pH

(3.6) was fixed. After 90 min, the reactions were completed by heating specimens for 10 min at 90°C. The hydrolysates were then subsequently centrifuged to isolate the precipitate at 7000 rpm, at 4°C for 30 min. Then, the temperature was maintained to −20°C for the storage of supernatant and was later freeze-dried and stored at 4°C.

2.5 | Determination of TPC

Total polyphenol contents (TPCs) of hyacinth flour, HPC and HPH were estimated according to method of Roy et al. (2020), with a slight modification. One milliliter of sample aliquot was added to distilled water (1.5 ml) and subsequently mixed with 0.1 M Folin–Ciocalteu reagent (0.5 ml), and the substances were then completely stirred. About 1 ml of Na_2CO_3 solution (20%) was mixed with the previous mixture after 1 min. The absorbance was measured at 750 nm after 30 min of incubation at 37°C. The calculation of total phenolics was estimated in gallic acid equivalent (GAE) using standard curve of gallic acid.

$$Y = 0.009X - 0.0109,$$

where $R^2 = 0.999$, Y is the absorbance of the sample tested, and X is the concentration obtained from standard curve.

2.6 | Determination of antioxidant activity

The scavenging effect of hyacinth flour, HPC, HPH, and AA samples was examined according to the technique reported by Yen and Chen (1995). A 2.0-ml test sample was applied to 0.16 mM DPPH (2.0 ml) methanolic solution. The solution was stirred for 45 s and then permitted to stand at ambient temperature for half an hour in the dark, and finally, absorbance was measured at 517 nm. The potential to scavenge the DPPH radical was estimated using the following formula:

$$\text{Scavenging effect}\,(\%) = [1 - (A_s - A_c)/A_c] \times 100,$$

where A_s and A_c are the absorbances of the sample and the control, respectively.

The DPPH free radical scavenging activity was expressed as IC_{50} value. The IC_{50} values for the sample concentration (mg/ml) required to inhibit of the 50% DPPH radical scavenging activity (DRSA) were calculated from the above equation.

2.7 | Application of hydrolysates to apple juice

2.7.1 | Preparation of juice sample

Apple juice was prepared according to Glevitzky et al. (2008) with some modification. Apples were bought from the local market, Sylhet, Bangladesh. Apples were crushed in a blender by mixing with 1000-ml distilled water, where the volume of apple concentrate was 165 ml/L. The sample was then split evenly into three different conical flasks. One of these was used as a control sample. The HPH extracted by pepsin and the HPC were independently blended with the remaining two samples at a rate of 3 g/L. Finally, all the samples were pasteurized at 90°C for 30 s and kept for 6 days at ambient temperature.

2.7.2 | Measurement of oxidizing substances

The oxidizing substances were evaluated according to the technique described in Sarker et al. (2020) with slight changes. A 20-ml sample of clear juice, 4 ml of glacial acetic acid, and 1 g of potassium iodide was added together in a 250-ml conical flask, and finally, 1-ml 2% starch solution was also mixed. The sample was vortexed using a vortex machine and kept in the dark for 30 min. The sample was then reacted with sodium thiosulfate (0.002 M) until the color of the starch-iodide had faded. A blank determination was also performed. A maximum amount of 1.4 ml of 0.002 M $Na_2S_2O_3$ was required (0.002%, calculated as H_2O_2) where 1 ml of 0.002 M $Na_2S_2O_3$ is equivalent to 0.032 mg of oxidizing substances, calculated as H_2O_2 (Roy et al., 2020).

2.8 | Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the data, using SPSS version 17 (SPSS, Inc., Chicago, Illinois, USA). Means were separated for comparison by Duncan's multiple range test (DMRT), and the statistical significance was defined as $p < 0.05$.

3 | RESULT AND DISCUSSION

3.1 | Proximate composition of hyacinth bean flour

The proximate composition of hyacinth bean seed powder (dry basis) was presented in Table 1. It contained 24.06% protein, 59.62%

TABLE 1 Proximate composition of hyacinth bean flour and crude protein

Components	Hyacinth bean flour (%)	Protein concentrate (%)
Protein	$24.06 \pm 2.13_b$	$76.56 \pm 2.63_a$
Carbohydrate	$59.62 \pm 3.27_a$	$17.87 \pm 3.45_b$
Fat	$2.94 \pm 0.35_a$	$1.01 \pm 0.13_b$
Ash	$4.22 \pm 0.27_a$	$2.03 \pm 0.28_b$
Moisture	$9.16 \pm 0.52_a$	$2.56 \pm 0.41_b$

Note: Values are mean ± standard deviations of three replicates. Means in a row with different letters are significantly different ($p < 0.05$) by DMRT.

carbohydrates, 2.94% fat, 4.22% ash, and 9.16% moisture content. Hossain et al. (2016) found similar data except ash and fat content which were 3.50 and 1.02, respectively. The ash content of mung bean was 3.64% to 4.24% reported by Li et al. (2010), and the fat content observed for *Phaseolus vulgaris* was 1.27% to 3.02% (Shimelis & Rakshit, 2005). These variations were possibly due to the form of soil wherein the bean species was cultivated, soil nutrient absorption ability of plants, environmental conditions, or genetic diversity. The protein content of hyacinth flour was almost identical to the results calculated from several other legumes, including such jack bean (23% to 30%) (Akpapunam & Sefa-Dedeh, 1997). In our previous work, the kidney bean contained 6.25% moisture content (Sarker et al., 2020). The activity of the microbes could be limited because of the very low moisture content of hyacinth bean found in this study, and therefore, the shelf life of the bean can be increased.

3.2 | Proximate composition of crude protein

The proximate composition of crude protein (dry basis) isolated from hyacinth bean was exhibited in the Table 1. It contained protein 76.56%, carbohydrates 17.87%, fat 1.01%, ash 2.03%, and moisture 2.56%. Because the product obtained by protein isolation contains 76.56% protein, it could be referred as protein concentrate (Boye et al., 2010). The protein content of HPC was roughly three times greater than hyacinth bean flour. However, the protein content of the HPC in the present study was lower than the HPC which was previously reported to be 89.9% to 94.4% in chickpea (Clemente et al., 1999). In this analysis, the reduced amount of protein content of HPC produced from hyacinth bean flour was mostly attributed to species variations, separation technique, and higher raw flour fat content. Due to the development of emulsion in combination with protein during extraction, such a high fat content may have decreased the effectiveness of protein extraction from raw flour. The amount of protein content for the HPC was similar to field pea's 77% (Tian et al., 1999) and greater than the Azufrado bean's concentrate which were 61% to 66% (Valdez-Ortiz et al., 2012). The fat content of HPC was similar to 1.1% in chickpea protein isolate (Clemente et al., 1999). The reduced fat content suggested that the protein concentrate's fat portion was mostly eliminated during the processing of the HPC. But the presence of fat in our study might be due to the fact that flour was not defatted initially. The ash content of HPC was close to that of mung bean protein concentrates which was 2.19% to 3.04% (Li et al., 2010) but much lower than flour, which may be due to the removal of most minerals in the supernatant after protein precipitation. The moisture content was very low that indicated that the HPC had been dried efficiently.

3.3 | Total polyphenol content

Beans contain phenolic compounds such as chlorogenic acid, catechin, anthocyanins, flavonoids, and tannins (Gan et al., 2017). While HPC preparation, certain polyphenols react with the proteins. Therefore, these polyphenols will be included in the final HPC. TPCs of hyacinth bean flour, HPC, and HPH are shown as mg GAE/g in Figure 1. In our experiment, TPC of the bean flour was 14.92 mg GAE/g which is almost similar to the polyphenol content (12.09 mg GAE/g) in hyacinth bean flour reported by Lin et al. (2014). TPC for some cereals and legumes varied 13.2 to 50.7 mg GAE/g and 17.0 to 21.9 mg GAE/g correspondingly (Djordjevic et al., 2011). But Kamath et al. (2015) found lower TPC in hyacinth bean flour which was 0.44 mg GAE/g. The possible explanation for the differences might be due to different variety and/or cultivation conditions, different methods of phenolic extraction and determination, and the choice of standard curve. In our study, the TPC of HPC (22.03 mg GAE/g) was significantly ($p < 0.05$) greater compared with bean flour. About 47.65% of TPC was increased in HPC. It indicated that a strong relationship existed between proteins and polyphenols. However, Tang et al. (2009) demonstrated that the TPC was remarkably greater for buckwheat protein isolate than for flour. Nonetheless, TPC of HPH (35.15 mg GAE/g) was considerably ($p < 0.05$) greater compared with that of flour (14.92 mg GAE/g) and HPC (22.03 mg GAE/g). Enzymatic hydrolysis of the protein-polyphenol complexes may have released the polyphenols bound in the peptide fragments, resulting in increased TPC in the HPH (Tang et al., 2009). An increase of about 59.56% of TPC was observed in HPH relative to HPC. Because hydrolysis induces maximum protein degradation, the scavenging effect of polyphenol complexes results in the conversion of polyphenol-associated peptide fragments.

3.4 | DPPH radical scavenging activity

Figure 2 displays the DPPH radical scavenging capacity of hyacinth flour, HPC, HPH, and industrial antioxidant AA at several strength (0.0032–10 mg/ml). The DPPH radical scavenging capacities were augmented with increasing HPH concentrations from 38.97% to 94.56%. These results were in agreement with Jao and Ko (2002) who

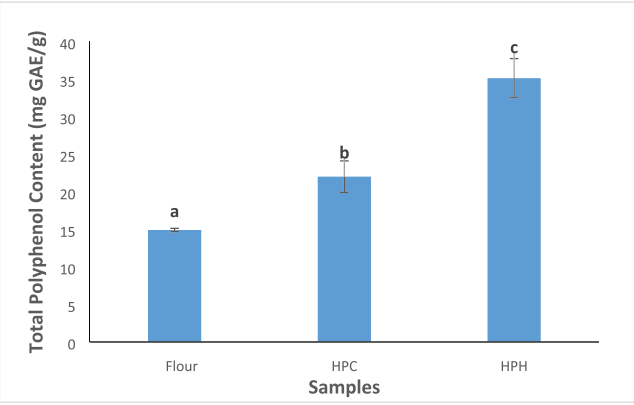

FIGURE 1 Total polyphenol content of hyacinth bean flour, hyacinth bean protein concentrate (HPC), and hyacinth bean protein hydrolysate (HPH)

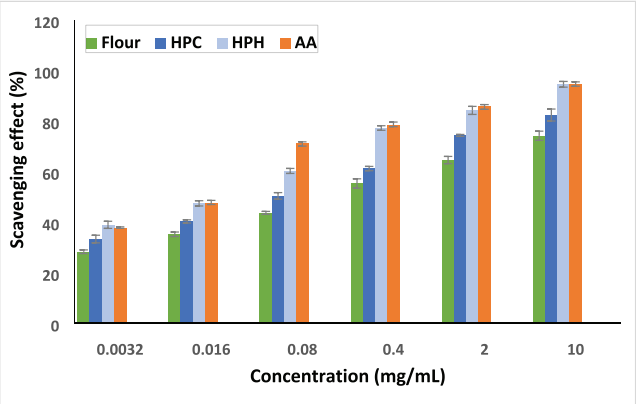

FIGURE 2 DPPH radical scavenging activity of hyacinth bean flour, hyacinth bean protein concentrate (HPC), hyacinth bean protein hydrolysate (HPH), and ascorbic acid (AA)

demonstrated that when the concentration increased, the DPPH activity from tuna cooking juice augmented from 17% to 75%. In wheat germ protein hydrolysates, similar phenomena were identified (Cheng et al., 2006). In this study, the DPPH activities of HPH were considerably ($p < 0.05$) greater compared with that of flour and HPC at same concentration. For example, at concentration 0.4 mg/ml, the antioxidant activity of flour, HPC, and HPH was found 55.32%, 61.23%, and 77.27%, respectively. The enhancement in antioxidant activity in the HPH was linked to the fragmentation of high molecular weight protein compounds (>50 kDa) into smaller size components caused by pepsin hydrolysis (Evangelho et al., 2016; Sarker et al., 2020). That results clearly indicated that the HPH probably contained some amino acids and peptides which became released from intact protein after hydrolysis. These amino acids and certain peptides were engaged in the disposal of electrons/hydrogen and were capable to react with free radicals to transform more unchanging products and to bring an end to radical chain reactions. Previously, Udenigwe and Aluko (2012) also explained that a large quantity of peptide and amino acid remainders has the capacity to pass electrons to free radicals that subsequently lead to a rise in the antioxidant activities of protein hydrolysates. As the polyphenol, indole, and imidazole serve as proton donors, the occurrence of aromatic amino acids in the sulfate group can enhance this function (Luna-Vital et al., 2015). The hydrophobic amino acids (e.g., val or leu) containing protein hydrolysates and peptides enhance hydrophobicity resulted in increasing their solubility in lipid as a result increases their antioxidant capacity (Saiga et al., 2003). Moreover, it was shown that HPC also exhibited DRSA. This might be due to their capacity to serve as a radical trapping mechanism and the existence of phenolics.

In the present study, there were no considerable ($p > 0.05$) differences between HPH and AA at the same concentration in most cases. This also indicated that HPH had remarkable antioxidant activities. It was found that at 2- and 0.4-mg/ml strength, the HPH exhibited DPPH activities of 84.23% and 77.27%, respectively. Previously, soybean protein hydrolysates exhibited scavenging activities 70% at 1.45 mg/ml (Abu-Salem et al., 2013), and almost similar results

TABLE 2 IC_{50} value of hyacinth flour, HPC, HPH, and AA

Samples	IC_{50} (mg/ml)
Hyacinth flour	$0.94 \pm 0.32_c$
HPC	$0.32 \pm 0.12_b$
HPH	$0.05 \pm 0.01_a$
AA	$0.02 \pm 0.01_a$

Note: Values are mean ± standard deviations of three replicates. Means in a column with different letters are significantly different ($p < 0.05$) by DMRT.
Abbreviations: AA, ascorbic acid; HPC, hyacinth bean protein concentrate; HPH, hyacinth bean protein hydrolysate.

reported by Sakanaka and Tachibana (2006) that protein hydrolysates of egg yolk showed 74.2% DRSA at 0.5-mg/ml concentration. The Flavourzyme and alcalase hydrolysates demonstrated a scavenging activity of 44% to 70% and 48% to 58% at 0.5 mg/ml, respectively (Bamdad et al., 2011). On the other hand, pea (Humiski & Aluko, 2007) exhibited up to 11% activities. The differences in DPPH antioxidant activity might be due to the difference in the composition of the amino acids of peptides and their placement in the chain of peptides inside protein hydrolysates.

3.5 | IC_{50} value of DRSA

The value of IC_{50} was considered as an efficient peptide concentration that was needed to scavenge 50% of the radical activity. The lower the IC_{50} value, the higher the dose response activity. The HPH showed significantly lower IC_{50} value (0.05 mg/ml) than IC_{50} (0.94 mg/ml) of flour but almost similar with AA (IC_{50} 0.02 mg/ml) (Table 2). That is why HPH is a very strong antioxidant, so it can cause 50% scavenging at a very low concentration. There is also considerable ($p > 0.05$) variation among flour and HPC. Therefore, it was suggested that HPC also has reasonable antioxidant property.

3.6 | Application of hydrolysates to apple juice

In order to investigate their antioxidant effects, HPC and HPH (3 g/L) were added to apple juice that was kept at ambient temperature for 6 days. In juice samples, they were capable to delay the development of oxidizing compounds. According to Figure 3, the oxidizing substances increased with the increase of storage period but the addition of HPH in apple juice reduced the oxidizing substances significantly in each storage period. At the initial stages of storage, it was found that the HPH prevented the development of oxidizing compounds significantly ($p < 0.05$) than control and HPC. The HPH retarded initiation of oxidizing compounds by 79.58% and 65.1% at Days 0 and 1, respectively. Likewise, it was observed that the quantity of oxidizing compounds in the control sample and HPC increased dramatically ($p < 0.05$) relative to HPH at the termination of storage period (6 days). In 6 days' time, the HPH prevented the development of oxidizing

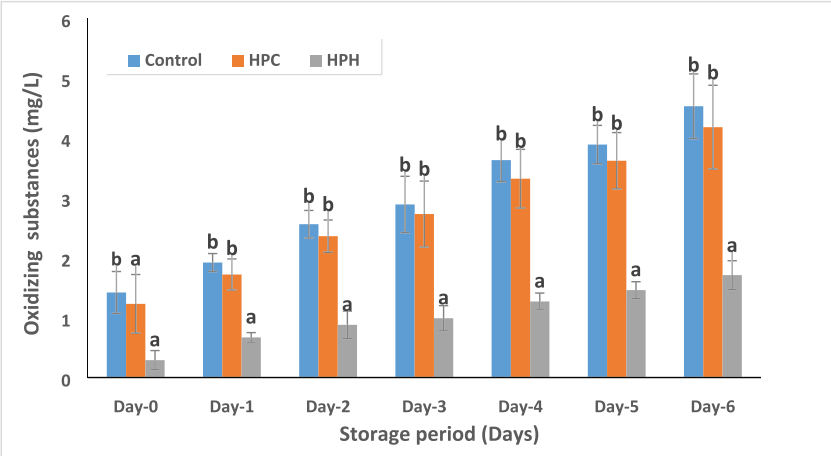

FIGURE 3 Inhibition of oxidizing substances formation by control, hyacinth bean protein concentrate (HPC), and hyacinth bean protein hydrolysate (HPH)

compounds 62.03% than control (Figure 3). The apple juice has lower antioxidant capacity (IC_{50} value: 2.5 mg/ml) and low TPC (5.85 mg GAE/g) than other common fruit juices (Beh et al., 2012). It also possesses a low quantity of vitamin C (Glevitzky et al., 2008) which declined significantly during heat processing such as pasteurization. Due to the low inherent antioxidant capacities of apple juice, the development of oxidizing compounds was higher with the advent of storage period. Therefore, the ability of apple juice to retard oxidation was decreased day by day. At the end of 6-day storage, concentration of oxidizing substances was augmented in control sample and the sample containing HPC than that of the sample containing HPH. This effect suggested that HPH can hinder the oxidation proficiently.

The reduction of oxidizing products by HPH might be due to bioactive peptides possessing antioxidant activity that are produced during enzymatic hydrolysis (Roy et al., 2020). In another study, about 2% protein hydrolysates of whey and soy were added to patties for a week in the previous report, which also successfully delayed oxidation (Peña-Ramos et al., 2004). The cooked ground beef prevented the development of thiobarbituric acid by 75% and 39% resulting from the application of 5% caseino-phosphorpeptides and casein hydrolysates at 4 days of storage period at 4°C (Díaz & Decker, 2004). Moreover, Wang and Xiong (2005) reported that 4% protein hydrolysates of potato which were added to beef patties and stored for a week at 4°C where potato protein hydrolysates reduced TBARS by 50.3% and decreased peroxide value by 91.7%. Furthermore, protein hydrolysates of egg yolk retarded TBARS formation by 43.8% in ground beef and 65.7% in tuna homogenates (Sakanaka & Tachibana, 2006). As in previous studies, HPH efficiently prevented the production of oxidizing compounds in apple. However, the degree of inhibition was not the same. It might be due to (1) variations in storage parameters, storage period, and processes of determination of oxidizing substances and (2) the application of several types of hydrolysates on different foods.

4 | CONCLUSION

The hyacinth bean is a high-protein food containing numerous bioactive amino acids. Pepsin hydrolysis of hyacinth bean increased the total polyphenol concentration in the hydrolysates and therefore, resulted in higher antioxidant activity in the hydrolysates than the protein concentrate. Furthermore, the hydrolysates had stronger antioxidant activity compared with AA. The hydrolysates also significantly prevented the development of oxidizing compounds in apple juice relative to control and protein concentrates. The outcomes of the present study demonstrate that hydrolysates may be effectively used as natural antioxidant and food additives in food industries.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

American Association of Cereal Chemists (AACC). (Ed.) (2000). *Approved methods of the AACC* (10th ed.). St. Paul, MN: The Association.

Abu-Salem, F. M., Mahmoud, M. H., El-Kalyoub, M., Gibriel, A., & Abou-Arab, A. (2013). Characterization of antioxidant peptides of soybean protein hydrolysate. *World Academy of Science, Engineering and Technology*, *7*, 522–526. https://doi.org/10.5281/zenodo.1086947

Akpapunam, M., & Sefa-Dedeh, S. (1997). Some physicochemical properties and anti-nutritional factors of raw, cooked and germinated Jack bean (*Canavalia ensiformis*). *Food Chemistry*, *59*(1), 121–125. https://doi.org/10.1016/S0308-8146(96)00248-8

Alam, M., Hossain, M., & Sarkar, A. (2020). Effect of edible coating on functional properties and nutritional compounds retention of air dried green banana (*Musa sapientum* L.). *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, *14*(2), 51–58.

Association of Official Analytical Chemists (AOAC). (Ed.) (1995). *Official methods of analysis* (15th ed.). Washington, DC: The Association.

Bamdad, F., Wu, J., & Chen, L. (2011). Effects of enzymatic hydrolysis on molecular structure and antioxidant activity of barley hordein. *Journal of Cereal Science*, *54*(1), 20–28. https://doi.org/10.1016/j.jcs.2011.01.006

Beh, L. K., Zakaria, Z., Beh, B. K., Ho, W. Y., Yeap, S. K., & Alitheen, N. B. M. (2012). Comparison of total phenolic content and antioxidant activities of freeze-dried commercial and fresh fruit juices. *Journal of Medicinal Plant Research*, *6*(48), 5857–5862. https://doi.org/10.5897/JMPR11.991

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International*, *43*(2), 414–431. https://doi.org/10.1016/j.foodres.2009.09.003

Carrasco-Castilla, J., Hernández-Álvarez, A. J., Jiménez-Martínez, C., Jacinto-Hernández, C., Alaiz, M., Girón-Calle, J., Vioque, J., & Dávila-Ortiz, G. (2012). Antioxidant and metal chelating activities of peptide fractions from phaseolin and bean protein hydrolysates. *Food Chemistry*, *135*(3), 1789–1795. https://doi.org/10.1016/j.foodchem.2012.06.016

Cheng, Y.-H., Wang, Z., & Xu, S.-Y. (2006). Antioxidant properties of wheat germ protein hydrolysates evaluated in vitro. *Journal of Central South University of Technology*, *13*(2), 160–165. https://doi.org/10.1007/s11771-006-0149-7

Clemente, A., Vioque, J., Sánchez-Vioque, R., Pedroche, J., Bautista, J., & Millán, F. (1999). Protein quality of chickpea (*Cicer arietinum* L.) protein hydrolysates. *Food Chemistry*, *67*(3), 269–274. https://doi.org/10.1016/S0308-8146(99)00130-2

Díaz, M., & Decker, E. A. (2004). Antioxidant mechanisms of caseinophosphopeptides and casein hydrolysates and their application in ground beef. *Journal of Agricultural and Food Chemistry*, *52*(26), 8208–8213. https://doi.org/10.1021/jf048869e

Djordjevic, T. M., Šiler-Marinkovic, S. S., & Dimitrijevic-Brankovic, S. I. (2011). Antioxidant activity and total phenolic content in some cereals and legumes. *International Journal of Food Properties*, *14*(1), 175–184. https://doi.org/10.1080/10942910903160364

Evangelho, J. A. D., Berrios, J. D. J., Pinto, V. Z., Antunes, M. D., Vanier, N. L., & Zavareze, E. D. R. (2016). Antioxidant activity of black bean (*Phaseolus vulgaris* L.) protein hydrolysates. *Food Science and Technology*, *36*, 23–27. https://doi.org/10.1590/1678-457X.0047

Gan, R. Y., Wang, M. F., Lui, W. Y., Wu, K., Dai, S. H., Sui, Z. Q., & Corke, H. (2017). Diversity in antioxidant capacity, phenolic contents, and flavonoid contents of 42 edible beans from China. *Cereal Chemistry*, *94*(2), 291–297. https://doi.org/10.1094/CCHEM-03-16-0061-R

Glevitzky, M., Pop, M., Brusturean, G.-A., Bogdan, I., Calisevici, M., & Perju, D. (2008). Efficient use of antioxidants to preserve fruit juice. *Revista de Chimie*, *59*, 1291–1295.

Halliwell, B., Murcia, M. A., Chirico, S., & Aruoma, O. I. (1995). Free radicals and antioxidants in food and in vivo: What they do and how they work. *Critical Reviews in Food Science and Nutrition*, *35*(1–2), 7–20. https://doi.org/10.1080/10408399509527682

Hossain, M., Arafat, M., Alam, M., & Hossain, M. (2021). Effect of solvent types on the antioxidant activity and total flavonoids of some Bangladeshi legumes. *Food Research*, *5*(4), 329–335. https://doi.org/10.26656/fr.2017.5(4).035

Hossain, S., Ahmed, R., Bhowmick, S., Al Mamun, A., & Hashimoto, M. (2016). Proximate composition and fatty acid analysis of *Lablab purpureus* (L.) legume seed: Implicates to both protein and essential fatty acid supplementation. *Springerplus*, *5*(1), 1–10. https://doi.org/10.1186/s40064-016-3587-1

Humiski, L., & Aluko, R. (2007). Physicochemical and bitterness properties of enzymatic pea protein hydrolysates. *Journal of Food Science*, *72*(8), S605–S611. https://doi.org/10.1111/j.1750-3841.2007.00475.x

Jao, C.-L., & Ko, W.-C. (2002). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. *Fisheries Science*, *68*(2), 430–435. https://doi.org/10.1046/j.1444-2906.2002.00442.x

Je, J.-Y., Park, P.-J., & Kim, S.-K. (2005). Antioxidant activity of a peptide isolated from Alaska pollack (*Theragra chalcogramma*) frame protein hydrolysate. *Food Research International*, *38*(1), 45–50. https://doi.org/10.1016/j.foodres.2004.07.005

Je, J.-Y., Qian, Z.-J., Byun, H.-G., & Kim, S.-K. (2007). Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. *Process Biochemistry*, *42*(5), 840–846. https://doi.org/10.1016/j.procbio.2007.02.006

Kamath, S. D., Arunkumar, D., Avinash, N. G., & Samshuddin, S. (2015). Determination of total phenolic content and total antioxidant activity in locally consumed food stuffs in Moodbidri, Karnataka, India. *Advances in Applied Science Research*, *6*(6), 99–102.

Korhonen, H., & Pihlanto, A. (2006). Bioactive peptides: Production and functionality. *International Dairy Journal*, *16*(9), 945–960. https://doi.org/10.1016/j.idairyj.2005.10.012

Lajolo, F. M., & Genovese, M. I. (2002). Nutritional significance of lectins and enzyme inhibitors from legumes. *Journal of Agricultural and Food Chemistry*, *50*(22), 6592–6598. https://doi.org/10.1021/jf020191k

Li, W., Shu, C., Yan, S., & Shen, Q. (2010). Characteristics of sixteen mung bean cultivars and their protein isolates. *International Journal of Food Science & Technology*, *45*(6), 1205–1211. https://doi.org/10.1111/j.1365-2621.2010.02259.x

Lin, K., Yeh, H., Lin, S., Yang, C., Tsai, S., Tsai, J., & Chao, P.-Y. (2014). Antioxidant activities of methanol extracts from selected Taiwanese herbaceous plants. *Journal of Food and Nutrition Research*, *2*(8), 435–442. https://doi.org/10.12691/jfnr-2-8-2

Luna-Vital, D. A., Mojica, L., de Mejía, E. G., Mendoza, S., & Loarca-Piña, G. (2015). Biological potential of protein hydrolysates and peptides from common bean (*Phaseolus vulgaris* L.): A review. *Food Research International*, *76*, 39–50. https://doi.org/10.1016/j.foodres.2014.11.024

Masuda, T., Inaba, Y., Maekawa, T., Takeda, Y., Yamaguchi, H., Nakamoto, K., Kuninaga, H., Nishizato, S., & Nonaka, A. (2003). Simple detection method of powerful antiradical compounds in the raw extract of plants and its application for the identification of antiradical plant constituents. *Journal of Agricultural and Food Chemistry*, *51*(7), 1831–1838. https://doi.org/10.1021/jf026112m

McClements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science*, *65*(8), 1270–1282. https://doi.org/10.1111/j.1365-2621.2000.tb10596.x

Meisel, H. (2004). Multifunctional peptides encrypted in milk proteins. *BioFactors*, *21*(1–4), 55–61. https://doi.org/10.1002/biof.552210111

Morris, J. B. (2009). Morphological and reproductive characterization in hyacinth bean, *Lablab purpureus* (L.) sweet germplasm with clinically proven nutraceutical and pharmaceutical traits for use as a medicinal food. *Journal of Dietary Supplements*, *6*(3), 263–279. https://doi.org/10.1080/19390210903070830

Peña-Ramos, E. A., & Xiong, Y. L. (2003). Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Science*, *64*(3), 259–263. https://doi.org/10.1016/S0309-1740(02)00187-0

Peña-Ramos, E. A., Xiong, Y. L., & Arteaga, G. E. (2004). Fractionation and characterisation for antioxidant activity of hydrolysed whey protein. *Journal of the Science of Food and Agriculture*, *84*(14), 1908–1918. https://doi.org/10.1002/jsfa.1886

Piccolella, S., Fiorentino, A., Pacifico, S., D'Abrosca, B., Uzzo, P., & Monaco, P. (2008). Antioxidant properties of sour cherries (*Prunus cerasus* L.): Role of colorless phytochemicals from the methanolic extract of ripe fruits. *Journal of Agricultural and Food Chemistry*, *56*(6), 1928–1935. https://doi.org/10.1021/jf0734727

Roy, M., Imran, M. Z. H., Alam, M., & Rahman, M. (2021). Effect of boiling and roasting on physicochemical and antioxidant properties of dark

red kidney bean (*Phaseolus vulgaris*). *Food Research*, *5*(3), 438–445. https://doi.org/10.26656/fr.2017.5(3).673

Roy, M., Sarker, A., Azad, M. A. K., Shaheb, M. R., & Hoque, M. M. (2020). Evaluation of antioxidant and antimicrobial properties of dark red kidney bean (*Phaseolus vulgaris*) protein hydrolysates. *Journal of Food Measurement and Characterization*, *14*(1), 303–313. https://doi.org/10.1007/s11694-019-00292-4

Rui, X., Boye, J. I., Ribereau, S., Simpson, B. K., & Prasher, S. O. (2011). Comparative study of the composition and thermal properties of protein isolates prepared from nine *Phaseolus vulgaris* legume varieties. *Food Research International*, *44*(8), 2497–2504. https://doi.org/10.1016/j.foodres.2011.01.008

Rui, X., Boye, J. I., Simpson, B. K., & Prasher, S. O. (2013). Purification and characterization of angiotensin I-converting enzyme inhibitory peptides of small red bean (*Phaseolus vulgaris*) hydrolysates. *Journal of Functional Foods*, *5*(3), 1116–1124. https://doi.org/10.1016/j.jff.2013.03.008

Saiga, A., Tanabe, S., & Nishimura, T. (2003). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry*, *51*(12), 3661–3667. https://doi.org/10.1021/jf021156g

Sakanaka, S., & Tachibana, Y. (2006). Active oxygen scavenging activity of egg-yolk protein hydrolysates and their effects on lipid oxidation in beef and tuna homogenates. *Food Chemistry*, *95*(2), 243–249. https://doi.org/10.1016/j.foodchem.2004.11.056

Sakanaka, S., Tachibana, Y., Ishihara, N., & Juneja, L. R. (2004). Antioxidant activity of egg-yolk protein hydrolysates in a linoleic acid oxidation system. *Food Chemistry*, *86*(1), 99–103. https://doi.org/10.1016/j.foodchem.2003.08.014

Sarkar, A., Ahmed, T., Alam, M., Rahman, S., & Pramanik, S. K. (2020). Influences of osmotic dehydration on drying behavior and product quality of coconut (*Cocos nucifera*). *Asian Food Science Journal*, *15*(3), 21–30. https://doi.org/10.9734/AFSJ/2020/v15i330153

Sarkar, A., Rahman, M. M., Sarkar, J. K., Alam, M., & Rashid, M. H. (2020). Growth and quality parameters of tea (*Camellia sinensis*) mediated by arbuscular mycorrhizal fungi. *Nova Biotechnologica et Chimica*, *19*(2), 232–239. https://doi.org/10.36547/nbc.v19i2.673

Sarkar, A., Rahman, S., Roy, M., Alam, M., Hossain, M., & Ahmed, T. (2021). Impact of blanching pretreatment on physicochemical properties, and drying characteristics of cabbage (*Brassica oleracea*). *Food Research*, *5*(2), 393–400. https://doi.org/10.26656/fr.2017.5(2).556

Sarker, A., Chakraborty, S., & Roy, M. (2020). Dark red kidney bean (*Phaseolus vulgaris* L.) protein hydrolysates inhibit the growth of oxidizing substances in plain yogurt. *Journal of Agriculture and Food Research*, *2*, 100062. https://doi.org/10.1016/j.jafr.2020.100062

Sathivel, S., Bechtel, P., Babbitt, J., Smiley, S., Crapo, C., Reppond, K., & Prinyawiwatkul, W. (2003). Biochemical and functional properties of herring (Clupea harengus) byproduct hydrolysates. *Journal of Food Science*, *68*(7), 2196–2200. https://doi.org/10.1111/j.1365-2621.2003.tb05746.x

Shaahu, D., Kaankuka, F., & Okpanachi, U. (2015). Proximate, amino acid, anti-nutritional factor and mineral composition of different varieties of raw lablab purpureus seeds. *International Journal of Scientific & Technology Research*, *4*, 157–161.

Shahidi, F. (2000). Antioxidants in food and food antioxidants. *Food/Nahrung*, *44*(3), 158–163. https://doi.org/10.1002/1521-3803(20000501)44:3<158::AID-FOOD158>3.0.CO;2-L

Shimelis, E. A., & Rakshit, S. K. (2005). Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *LWT-Food Science and Technology*, *38*(4), 331–338. https://doi.org/10.1016/j.lwt.2004.07.002

Subagio, A. (2006). Characterization of hyacinth bean (*Lablab purpureus* (L.) sweet) seeds from Indonesia and their protein isolate. *Food Chemistry*, *95*(1), 65–70. https://doi.org/10.1016/j.foodchem.2004.12.042

Suetsuna, K. (2000). Antioxidant peptides from the protease digest of prawn (*Penaeus japonicus*) muscle. *Marine Biotechnology*, *2*(1), 5–10. https://doi.org/10.1007/s101269900002

Tang, C.-H., Peng, J., Zhen, D.-W., & Chen, Z. (2009). Physicochemical and antioxidant properties of buckwheat (*Fagopyrum esculentum* Moench) protein hydrolysates. *Food Chemistry*, *115*(2), 672–678. https://doi.org/10.1016/j.foodchem.2008.12.068

Tian, S., Kyle, W. S., & Small, D. M. (1999). Pilot scale isolation of proteins from field peas (*Pisum sativum* L.) for use as food ingredients. *International Journal of Food Science & Technology*, *34*(1), 33–39. https://doi.org/10.1046/j.1365-2621.1999.00236.x

Udenigwe, C. C., & Aluko, R. E. (2012). Food protein-derived bioactive peptides: Production, processing, and potential health benefits. *Journal of Food Science*, *77*(1), R11–R24. https://doi.org/10.1111/j.1750-3841.2011.02455.x

Valdez-Ortiz, A., Fuentes-Gutiérrez, C. I., Germán-Báez, L. J., Gutiérrez-Dorado, R., & Medina-Godoy, S. (2012). Protein hydrolysates obtained from Azufrado (sulphur yellow) beans (*Phaseolus vulgaris*): Nutritional, ACE-inhibitory and antioxidative characterization. *LWT-Food Science and Technology*, *46*(1), 91–96. https://doi.org/10.1016/j.lwt.2011.10.021

Velioglu, Y., Mazza, G., Gao, L., & Oomah, B. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, *46*(10), 4113–4117. https://doi.org/10.1021/jf9801973

Vijayabaskar, P., & Shiyamala, V. (2012). Antioxidant properties of seaweed polyphenol from *Turbinaria ornata* (Turner) J. Agardh, 1848. *Asian Pacific Journal of Tropical Biomedicine*, *2*(1), S90–S98. https://doi.org/10.1016/S2221-1691(12)60136-1

Wang, J.-S., Zhao, M.-M., Zhao, Q.-Z., & Jiang, Y.-M. (2007). Antioxidant properties of papain hydrolysates of wheat gluten in different oxidation systems. *Food Chemistry*, *101*(4), 1658–1663. https://doi.org/10.1016/j.foodchem.2006.04.024

Wang, L. L., & Xiong, Y. L. (2005). Inhibition of lipid oxidation in cooked beef patties by hydrolyzed potato protein is related to its reducing and radical scavenging ability. *Journal of Agricultural and Food Chemistry*, *53*(23), 9186–9192. https://doi.org/10.1021/jf051213g

Wu, H.-C., Chen, H.-M., & Shiau, C.-Y. (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research International*, *36*(9–10), 949–957. https://doi.org/10.1016/S0963-9969(03)00104-2

Yen, G.-C., & Chen, H.-Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry*, *43*(1), 27–32. https://doi.org/10.1021/jf00049a007

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