ORIGINAL ARTICLE

Effect of dephenolization and pH on functional properties, amino acid profile, and nutritional characteristics of protein isolate from Meghalayan cherry (*Prunus nepalensis)* kernel

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Abstract

Stone fruit processing produces almost 20–30% of waste as seed kernels, which are rich source of valuable compounds like protein that can be used in various food formulations. So, the present study aimed to isolate protein from Meghalayan cherry kernel and study the effect of pH and dephenolization on biochemical, functional, and nutritional characteristics of protein isolate from the Meghalayan cherry kernel. Alkali (pH, 8.5–12.5) extraction and isoelectric precipitation were used to isolate kernel protein. Based on yield, purity, and color, pH 10.5 was most suitable for extracting protein. Dephenoized protein isolate (MCKPI-DP) showed higher protein content ($88.81 \pm 0.03\%$) as compared to natural protein isolate (MCKPI-N) ($83.50 \pm 0.01\%$). The significantly higher solubility, dispersibility, foaming properties, emulsion stability, and capacity were seen in dephenolized protein isolate, which increased with increased pH. Water binding capacity was higher for MCKPI-N than MCKPI-DP and further increased with an increase in pH. Meghalayan cherry kernel protein isolate was a rich source of essential amino acids except tryptophan required by infants and adults (FAO). MCKPI-DP showed better protein efficiency ratio ($2.67 \pm 0.03(PER_1)$, $2.49 \pm 0.02(PER_2)$, $2.14 \pm 0.04(PER_3)$), essential amino acid index (78.82 ± 0.85), EAA/TAA % (41.76 ± 0.01), nutritional index (65.82 ± 0.71), and biological value (74.22 ± 0.93) indicating the presence of good-quality proteins. As per the FAO standard, the amino acid score showed that all essential amino acids are in excess, thus helping fulfill essential amino acid requirements and being a good protein supplement source in food products. Moreover, this work helps better utilize agro-wastes as low-cost substrates for protein isolation.

Keywords *Prunus nepalensis* · Dephenolization · Protein isolate · Functional properties · Amino acids · Nutritional profile

1 Introduction

For many years, human protein nutrition has emphasized animal products as a source of protein. Animal protein is referred to as a complete protein source since it contains all necessary amino acids. The exponential rise of the human population raises demand for animal protein, which, in turn, restricts its supply. The scarcity of resources to meet demands gives rise to an alternate source of protein, which could provide the same health benefits and helps in eliminating animal-based product safety issues. Plant-based edible protein is the upcoming solution for such concerns [1]. Animal husbandry is also not much regarded as an environmentally friendly practice, and thus, it could be replaced by plant-based protein as these are cost-effective sources [2]. Agro-industrial by-products like fruit seeds are a rich source of proteins and other nutrients that humans can consume. Currently, these massive wastes are discarded without being recycled. Protein recovery and utilization for human consumption can help prevent environmental pollution and contribute to the industry's profit by lowering waste treatment costs.

Meghalayan cherry (*Prunus nepalensis*), locally called sohiong, belongs to the Rosaceae family in the Khasi and Jaintia hills of Meghalaya. It is consumed as raw and may be used to manufacture juices, jam, preserves, assorted wine, squash, etc. [3, 4]. With the industrial application,

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by-product generation also comes into consideration which causes disposal problems in the industry. The cherry kernels (approx. 30% of whole fruit) are a prominent source of protein to fulfill the industrial demand as an economical raw material for the replacement of expensive protein sources. Direct utilization of the kernel is limited as its sensory qualities are not appealing due to its dark color and bitter taste. The kernel of the fruit seed is a rich source of polyphenols which gives it different organoleptic properties [5]. The presence of polyphenols in kernel hinders the protein quality by reducing its digestibility, imparting unwanted brown color and structure alteration, which also affects the functional characteristics of protein in the different food systems [5]. Polyphenols are more prone to oxidation due to polyphenol oxidase and extraction conditions for protein extraction. The oxidation of polyphenols results in the production of compounds like o-quinones and o-dihydroxy structure, which are highly reactive and covalently attached to thiol and amino groups of protein. These bondings impart green and dark brown color to protein meal and isolates [6]. Hence, for the utilization as a source of protein, seed kernels must be first treated to remove the polyphenols.

The high quality and quantity of kernel proteins determine their good nutritional and functional properties. Apart from being a good source of essential amino acids, protein's performance as a good ingredient is determined by its functional qualities in various processing conditions, such as pH, temperature, and processing time. It has been observed that the functional characteristics of protein vary according to the change in pH. Thus, it is important to study the effects of pH on the functional characteristics of protein isolates as it can alter the properties of the end product [7]. Solubility is the crucial property of protein isolates, which helps determine other functional characteristics. Polyphenols significantly impact protein solubility as they enhance the internal cross-linking of protein, which reduces the net surface charge on protein molecules. This results in a reduction of protein solubility and ultimately affects the behavior of other functional properties.

The limited literature on nutritional and functional characteristics of Meghalayan cherry kernel protein and considering kernel proteins as an alternative source of protein to handle ever-increasing food security problems, the present study was intended to study the effect of dephenolization and pH on the biochemical and functional properties of the Meghalayan cherry kernel protein isolate. Furthermore, amino acid profiling, nutritional properties, and amino acid score for infants and adults according to FAO (2013) were also investigated.

2 Material and methods

2.1 Procurement of raw materials

Mature Meghalayan cherry fruits were collected during September 2019 from Shillong market, Meghalaya (India). The kernel was collected by cracking the seed shell and dried in a tray drier (SICO House, Patiala, India) at 45 ± 2 °C. Dried kernels were then milled in a laboratory mill (Agrosaw Pvt. Ltd. Haryana, India), and to obtain uniformity in particle size, kernel powder was sieved through a 60 mesh size sieve. Meghalayan cherry kernel powder was kept in airtight containers at 4 °C till analysis. Analytical grade reagents were used for the analysis and were procured from Sigma-Aldrich, Mumbai, India.

2.2 Sample preparation

The defatting of kernel powder was done by soxhlet extraction using hexane as a solvent for 5 h. The dried defatted kernel powder was subjected to detoxification by soaking (1:10 w/v) in water for 12 h, heating at 60 °C for 30 min, drained and dried in an oven at 45 ± 2 °C. The defatted and detoxified kernel powder was kept in airtight containers at 4 °C till analysis.

2.3 Phenol compound extraction

The conventional solvent extraction method was used to extract phenolic compounds from Meghalayan cherry kernel. The detoxified sample was dispersed in 60% (v/v) methanol in the ratio of 1:20 (w/v) and kept for 2 h with continuous stirring. The suspension obtained was filtered, and the extraction process was repeated four times. The dephenolized kernel powder thus obtained was then dried in a hot air oven overnight at 45 ± 2 °C. The two portions of cherry kernel powder with polyphenols and cherry kernel powder without polyphenols were obtained.

2.4 Isolation of protein and process standardization

Cherry kernel protein isolation was done by an alkaline extraction process followed by isoelectric precipitation [8]. Five different pH (8.5, 9.5, 10.5, 11.5, and 12.5) solutions of cherry kernel powder with polyphenols and cherry kernel powder without polyphenols were made with 2 N NaOH. The ratio of powder to solvent was kept at 1:10. The produced slurries were heated at 40 °C temperature with continuous stirring in a hot water bath for 2 h. After heating, the protein-containing slurry was centrifuged (Eppendorf, India) at $8000 \times g$ for 20 min, and

the supernatant was collected. The protein precipitation was carried out by inducing the collected supernatant at its isoelectric pH of 4.5 with 2 N HCl. The precipitated protein was collected by centrifugation process at $8000 \times g$ for 20 min, followed by freeze-drying (using freeze dryer (Allied frost, India) of the collected sample and kept in storage at 4 °C until further analysis. The protein isolated from the cherry kernel with polyphenols and without polyphenols was designated as MCKPI-N and MCKPI-DP. The standardization of protein isolate was done based on yield, purity, and color as many food manufacturers request protein isolates with high yield and purity with light color.

2.5 Determination of protein yield and purity

The yield of MCKPI-N and MCKPI-DP extracts acquired at various pH was calculated as per the method of Kaushik et al. [9] as follows:

$$Yield = \frac{P}{S} \times 100$$

where "P" is the weight (g) of protein isolate powder acquired after the extraction process and freeze-drying and "S" is the weight (g) of seeds powder obtained for the protein extraction. For determining the purity of protein isolates, the AOAC [10] method was adopted.

2.6 Color measurement

The color of protein isolates, MCKPI-N and MCKPI-DP, was measured by Hunter colorimeter, Model D25, optical Sensor (Hunter Associates Laboratory Inc., Reston, VA., USA) on account of CIE L*, a*, and b* values. A cell of glass carrying protein isolates was put above the source of light covered with a white plate and L*, a*, and b* color values were noted down.

2.7 Characterization of protein isolates

2.7.1 Chemical characteristics of protein isolates

To determine moisture, protein (nitrogen conversion factor is 6.25) and ash content of MCKPI-N and MCKPI-DP, the standard methods of AOAC [10] were used. The total phenolic content (TPC) was determined by Folin-Ciocalteu reagent method [11]. Gallic acid was taken as standard and absorbance was taken at 765 nm. The total phenol content was expressed as mg of gallic acid per g (mgGAE/g) of extract.

2.7.2 Wettability of protein isolates

Wettability of MCKPI-N and MCKPI-DP was ascertained by the static wetting test used by Mir et al. [8] with some modifications. A 250-ml beaker carrying 100 ml of distilled water was used and a glass funnel with its open end blocked by a test tube was kept above the beaker with the help of a ring stand. Protein isolate sample (1 g) was put in the funnel, and a test tube was taken out to allow the protein powder sample to fall inside the beaker and a stopwatch was used to record the time. Time was noted down for the powder sample to become thoroughly wet (when all the powder particles crept into the water's surface).

2.7.3 Effect of dephenolization and pH on functional properties of Meghalayan cherry kernel protein isolates

2.7.3.1 Solubility The solubility of MCKPI-N and MCKPI-DP was determined by Achouri et al. [12]. Protein isolate dispersions (2 g/100 ml) were prepared with distilled water at five different pH (3.5–11.5) and mixed for 1 h using a magnetic stirrer. The solution was then centrifuged at $3000 \times g$ for 30 min. The soluble protein contents present in the supernatant were measured by Kjeldhal method, and the following equation calculated the solubility:

$$Solubility\ (\%) = \frac{Amount\ of\ the\ protein\ in\ supernatent}{Amount\ of\ the\ protein\ in\ sample} \times 100$$

2.7.3.2 Emulsifying activity and emulsion stability Emulsifying activity (EA) and emulsion stability (ES) of MCKPI-N and MCKPI-DP were determined by the method of Lawal et al. [13] with slight modifications. Briefly, 5 ml of 2% (w/v) protein solution at different pH (3.5–11.5) was homogenized with 5 ml soybean oil. The emulsions were then centrifuged for 5 min at $3500 \times g$. The height of emulsified layer and that of the total contents in the tube was measured. The emulsifying activity (EA) was calculated as:

$$EA\ (\%) = \frac{Height\ of\ emulsified\ layer}{Height\ of\ total\ content\ in\ the\ tube} \times 100$$

For the estimation of emulsion stability, the samples were heated at 80 °C for 30 min. It was then centrifuged at $3500 \times g$ for 5 min and emulsion stability was calculated as:

$$ES\ (\%) = \frac{Height\ of\ emulsified\ layer}{Height\ of\ total\ content\ in\ the\ tube} \times 100$$

2.7.3.3 Water-binding capacity The procedure of Malik and Saini [14] based on the principle of quantitative indicators of the amount of water retained/entrapped within a protein matrix, was applied to estimate the water-binding

capacity of MCKPI-N and MCKPI-DP, with slight changes. Protein isolate (1 g) was mixed with 10 ml of distilled water, and the desired pH was adjusted to 3.5–11.5, followed by stirring for 5 min. The mixture was left to stand for 30 min and then centrifuged at $5000 \times g$ for 30 min. The supernatant was disposed of, and the tube was upturned for 25 min at 45 °C to siphon off the remaining water from the protein sediment. The following equation calculated the water-binding capacity:

$$WBC(\%) = \frac{a - b}{c} \times 100$$

where "a" is the weight (g) of the tube with the protein isolate and absorbed water. "b" is the weight (g) of the tube with protein isolate. "c" is the weight (g) of the protein isolate.

2.7.3.4 Dispersibility The procedure of Kullarni et al. [15] was applied to calculate the dispersibility of protein isolated with slight changes. Three grams each of MCKPI-N and MCKPI-DP were dispersed distinctly in 30 ml of distilled water in measuring cylinders, and pH (3.5–11.5) was adjusted either with 0.5 N NaOH or 0.5 N HCl. The mixtures were vigorously stirred and allowed to settle for 2 h. The volumes of settled particles acquired were measured, and dispersibility was calculated as per the following equation:

$$Dispersibility = \frac{Total\ volume - settled\ volume}{Total\ volume} \times 100$$

2.7.3.5 Foaming capacity and foam stability Foaming capacity and stability were calculated as per the procedure of Fekria et al. [16]. Briefly, 3 g of MCKPI-N and MCKPI-DP were dispersed in 100 ml distilled water and the solution pH was altered from 3.5 to 11.5. The solution was then whipped for 5 min using high-speed mixer blender (Remi, India Pvt. Ltd.). The whipped solution was replaced in a measuring cylinder and foam volume was instantly measured to determine the foaming capacity. The foam volume was measured after 15, 30, 45, and 60 min to calculate the volume change with time. The percentage foam volume that remained after each time interval depicted the foam stability after that time interval.

2.8 Amino acid composition

The hydrolysis of MCKPI-N and MCKPI-DP was done at 110 °C in 6 M HCl for 24 h and then allowed to cool to room temperature. The visible sediments in the hydrolysate were removed by filtration, and the dried residue was dissolved in citrate buffer after flash evaporation. A known sample volume was injected into an automatic amino acid analyser (Biochrom, USA) to estimate the amino acid profile in the samples. The analysis was carried out in triplicate, and the amino acid composition was reported in g/kg protein.

2.9 Estimation of nutritional quality of Meghalayan cherry kernel protein isolate

The composition of amino acids of MCKPI-N and MCKPI-DP were used for the estimation of various nutritional parameters studied as follows:

2.9.1 Protein effeciency ratio (PER)

Protein efficiency ratio (PER) was calculated by following three regression equations given by Alsmeyer et al. [17]:

$$PER_1 = -0.684 + 0.456 \text{ X Leu} - 0.047 \text{ X Pro}$$

$$PER_1 = -0.468 + 0.454 \text{ X Leu} - 0.105 \text{ X Tyr}$$

$$PER_3 = -1.816 + 0.435\ X\ Met + 0.78\ X\ Leu$$
$$+ 0.211\ X\ His - 0.944\ X\ Tyr$$

2.9.2 Essential amino acid index

Essential amino acid index (EAAI) was calculated based on amino acid composition standard of whole egg protein using the method of Oser [18] as per the equation:

$$EAAI = \sqrt{\frac{Lys_a \text{ X Tyr}_a \dots \dots \text{ X His}_a}{Lys_b \text{ X Tyr}_b \dots \dots \text{ X His}_b}}$$

where "a" is the amino acid in test sample and "b" is the amino acid in reference protein sample.

2.9.3 BV PER$_1$

Biological value was estimated as per the equation of Oser [18].

$$BV = 1.09 \times \text{Essential amino acid index} - 11.7$$

2.9.4 Nutritional Index

Nutritional index (NI) was estimated as a function of essential amino acid index and total protein, as used by Ijarotimi [19]:

$$NI = \frac{EAAI \times Protein(\%)}{100}$$

2.9.5 Amino acid score (chemical scoring)

Amino acid score was estimated as the ratio of observed value of essential amino acid to the reference pattern as provided by FAO [20].

$$\text{Amino acid score} = \frac{\text{Certain EAA content}}{\text{Recommended EAA content}} \times 100$$

2.10 Statistical analysis

A two-way analysis of variance test (ANOVA) was performed using the software (Statistica-7 (M/s. Stat Soft Inc., OK, USA) to evaluate the effect of pH and dephenolization on functional characteristics of cherry kernels protein isolates. Duncan's test determined significant differences between means at the 5% significance level. Data reported are mean ± SD of triplicate observation.

3 Results and discussion

3.1 Isolation of protein and process standardization

Table 1 shows the parameters used for the process standardization of protein isolate from Meghalayan cherry kernel. Protein isolate from Meghalayan cherry kernel without polyphenols showed higher yield and purity of protein isolate than cherry kernel with polyphenols. This might be related to the interaction of polyphenols with proteins at basic pH during the extraction process, which caused the decrease of protein content in the extracted protein isolates [17]. Similar results were found by Malik and Saini [14] and Subhashi et al. [21]. The yield of protein isolate for both MCKPI-N and MCKPI-DP increased with an increase in pH (8.5–12.5) and varied from 9.82 ± 0.02 to $15.21 \pm 0.02\%$ and 10.37 ± 0.04 to $16.08 \pm 0.03\%$, respectively. This was because of the fact that protein isolate's solubility gets raised towards higher alkaline conditions of the isolation medium. In higher alkaline conditions, the proteins tend to become more negatively charged because of the ionization of the carboxylic groups and de-protonation of the amine group, which increases the electrostatic repulsion between negatively charged proteins. Thus, increases the solubility of proteins with increase in pH values [22]. Given outcomes agrees with those of Mir et al. [8]. Even so, the purity of protein isolate decreased from 87.03 ± 0.02 to $77.25 \pm 0.03\%$ and 91.71 ± 0.04 to $80.15 \pm 0.02\%$ for MCKPI-N and MCKPI-DP, respectively, with an increase in pH. The protein content of MCKPI-N and MCKPI-DP was comparable to that of other protein isolates, i.e., 83.96% in cranberry bean protein isolate [23], 89.7% in soyabean protein isolate [24], and 89.42% in sunflower kernel protein isolate [14].

A significant difference was seen in color values for both MCKPI-N and MCKPI-DP and also with change in pH of solution. MCKPI-N showed lower L^* value but higher a^* and b^* value at all the pH for MCKPI-DP. This was because the interaction of phenols-protein and oxidation of phenolic components to o-quinones at basic pH during protein extraction imparts darker color to protein isolate. Whereas, increase in pH for both MCKPI-N and MCKPI-DP showed the highest value of L* (78.30 for CKPI-N and 81.09 for CKPI-DP) at pH 10.5 decreased further. The value of a^* and b^* varied from 2.01–4.02 and 14.22–19.72 for MCKPI-N and 2.12–3.97 and 10.36–17.15 MCKPI-DP, respectively. Similar results were reported by Salgado et al. [25] and Xu et al. [26] in case of sunflower protein concentrate and isolate and canola protein isolate, respectively. pH 10.5 was found to be suitable for extracting protein from Meghayalan cherry kernel based on desirable yield, purity of protein, and adequate color.

Table 1 Standardization of protein isolate from MCKPI-N and MCKPI-DP

pH	MCKPI-N					MCKPI-DP				
	Yield (%)	Purity (%)	Color			Yield (%)	Purity (%)	Color		
			L^*	a^*	b^*			L^*	a^*	b^*
$PI_{8.5}$	9.82 ± 0.02e	87.03 ± 0.02a	73.07 ± 0.32d	3.22 ± 0.25c	17.23 ± 0.60c	10.37 ± 0.04e	91.71 ± 0.04a	77.01 ± 0.42d	2.62 ± 0.28c	13.23 ± 0.16c
$PI_{9.5}$	11.54 ± 0.01d	85.68 ± 0.03b	76.94 ± 0.30b	3.94 ± 0.20b	18.61 ± 0.35b	12.72 ± 0.02d	90.35 ± 0.02b	79.55 ± 0.26b	3.42 ± 0.27b	15.77 ± 0.42b
$PI_{10.5}$	13.63 ± 0.02c	83.50 ± 0.01c	78.30 ± 0.39a	4.12 ± 0.29a	19.72 ± 0.21a	14.19 ± 0.02c	88.81 ± 0.03c	81.09 ± 0.39a	3.97 ± 0.14a	17.15 ± 0.31a
$PI_{11.5}$	14.50 ± 0.03b	78.43 ± 0.05d	75.77 ± 0.49c	2.44 ± 0.34d	16.12 ± 0.56d	15.42 ± 0.01b	82.50 ± 0.02d	78.35 ± 0.31c	2.37 ± 0.19d	11.25 ± 0.24d
$PI_{12.5}$	15.21 ± 0.02a	77.25 ± 0.03e	71.31 ± 0.42e	2.01 ± 0.37e	14.22 ± 0.39e	17.08 ± 0.03a	80.15 ± 0.02e	75.53 ± 0.40e	2.12 ± 0.19e	10.36 ± 0.28e

Values are in mean ± standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$) through Duncan's test

MCKPI-N natural Meghalayan cherry kernel protein isolate, *MCKPI-DP* dephenolized Meghalayan cherry kernel protein isolate, *PI_{8.5}* protein isolate at pH 8.5, *PI_{9.5}* protein isolate at pH9.5, *PI_{10.5}* protein isolate at pH10.5, *PI_{11.5}* protein isolate at pH 11.5, *PI_{12.5}* protein isolate at pH 12.5

3.2 Characterization of protein isolate

3.2.1 Chemical characteristics of natural and dephenolized Meghalayan cherry kernel protein isolate

Table 2 shows the chemical composition of protein isolate from MCKPI-N and MCKPI-DP. The extraction of Meghalayan cherry kernel with methanol significantly reduced the phenolic content from 3.86 ± 0.04 mgGAE/g DW to 0.19 ± 0 mgGAE/g DW, which showed 95% decrease in total phenolics. MCKPI-N ($83.50 \pm 0.01\%$) had lower protein content than MCKPI-DP ($88.81 \pm 0.03\%$) due to the interaction of polyphenols with proteins at basic pH 10.5 during extraction. Similar results were reported by Subasi et al. [21] and Malik and Saini [17] in case of sunflower kernel. Significant difference was also observed in the case of ash in which MCKPI-N showed higher values than MCKPI-DP.

3.2.2 Wettability of natural and dephenolized Meghalayan cherry kernel protein isolate

The dispersion of protein powders to make aqueous beverages depends on their wettability. Fluid can disperse or adhere to a solid surface surrounded by other fluids. Density, porosity, particle size, surface charge, surface area, and presence of amphipathic substances between the surface of the powder and penetrating fluid affects the wettability. Higher wettability was observed in the case MCKPI-DP (22 ± 0.90 min) as compared to MCKPI-N (18 ± 0.81 min). It might be because reducing polyphenols reduces the hydrophobicity of the protein, which results in proteins wetting faster [17].

3.2.3 Effect of dephenolization and pH on functional properties of protein isolate of Meghayalan cherry kernel

Solubility Solubility of protein is an important factor for food systems as it impacts the other functional characteristics such as emulsifying and foaming capacity and works as a convenient indicator of denaturation and interactivity of proteins. The solubility of a protein depends on pH, ionic strength, temperature, concentration, and the presence of other molecules [27]. A significant difference was seen in protein solubility for both MCKPI-N and MCKPI-DP with a change in pH. The lowest protein isolate solubility was seen at pH 5.5 near the isoelectric point and increased steadily on both sides of the pH. It might be because of no net charge (neutral charge) on protein at its isoelectric point, which results in that there is not any repulsive force between proteins which leads to protein–protein interaction and hence protein aggregates [28]. This aggregation of proteins leads

Table 2 Chemical composition of natural and dephenolized Meghayalan cherry kernel protein isolate

Sample	Moisture (%)	Protein (%)	Ash (%)	TPC (mgGAE/g)
MCKPI-N	8.73 ± 0.37a	83.50 ± 0.01b	2.64 ± 0.21a	3.86 ± 0.04a
MCKPI-DP	8.48 ± 0.43a	88.81 ± 0.03a	1.71 ± 0.39b	0.19 ± 0b

Values are in mean $\pm$ standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$) through Duncan's test

MCKPI-N natural Meghalayan cherry kernel protein isolate, *MCKPI-DP* dephenolized Meghalayan cherry kernel protein isolate, *TPC* total phenol content

to lesser solubility at isoelectric pH. At higher alkaline pH, the protein solubility increases as seen in Table 3. This occurred due to increased negative charge which will lead to repulsive electrostatic interactions between protein molecules which hinder their aggregation and hence increased solubility. Malik and Saini [14] also showed higher protein isolate solubility at pH 11 and lowest at pH 5 in the case of sunflower seed and kernel protein isolate.

In the case of MCKPI-N and MCKPI-DP, the protein isolate solubility was seen higher in cherry kernel without polyphenols than cherry kernel with polyphenols. After interactivity with phenolic components, the protein denaturation can be accredited to the decreasing solubility of modified proteins by phenols [29]. Moreover, the reaction of protein with phenolic components goes along with a corresponding blocking of hydrophilic groups and introducing an aromatic ring structure, resulting in the hydrophobic nature of protein and subsequent reduction in protein solubility [30].

Emulsion activity and stability During emulsion formation, the proteins are adsorbed in a tightly packed layer at the surface of oil droplets [31]. The emulsion activity of the protein is represented by absorption of protein on the oil–water interface, whereas its ability to form stable emulsion without flocculation represents the emulsion stability. The effect of dephenolization and pH on the emulsion activity and emulsion stability is seen in Table 3. Increase in the pH showed increased emulsion activity, with the highest value for MCKPI-N and MCKPI-DP was $41.17 \pm 0.53\%$ and $47.88 \pm 0.49\%$, respectively, at pH 11.5. Similar increase in emulsion activity with pH were shown by Xu et al. [32] and Ulloh et al. [33]. The same trend was seen in emulsion stability where emulsion stability varied from 36.59 ± 0.37 to $46.55 \pm 0.47\%$ for MCKPI-N and 37.66 ± 0.58 to $53.89 \pm 0.49\%$ for MCKPI-DP. The change in emulsion activity and emulsion stability of protein isolate with change

Table 3 Effect of dephenolization and pH on functional properties of Meghalayan cherry kernel protein isolate

pH	Solubility (%)		Emulsifying activity (%)		Emulsion stability (%)		Water binding capacity (%)		Dispersibility (%)	
	MCKPI-N	MCKPI-DP	MCKPI-N	MCKPI-DP	MCKPI-N	MCKPI-DP	MCKPI-N	MCKPI-DP	MCKPI-N	MCKPI-DP
3.5	31.25±0.67d	36.42±0.47d	33.30±0.49d	35.11±0.63d	36.59±0.37d	37.66±0.58d	214.65±0.59d	202.86±0.80d	63.28±0.64d	66.89±0.56d
55	25.87±0.58e	29.15±0.38e	31.58±0.44e	32.41±0.35e	35.78±0.47e	36.96±0.62e	208.32±0.63e	183.43±0.67e	60.44±0.80e	61.83±0.63e
7.5	48.92±0.59c	54.34±0.49c	35.41±0.41c	37.11±0.44c	39.02±0.60c	41.14±0.56c	220.97±0.69c	210.95±0.58c	69.90±0.69c	73.40±0.41c
9.5	56.47±0.41b	63.77±0.55b	40.02±0.32b	42.29±0.50b	43.20±0.38b	45.58±0.44b	238.15±0.52b	223.63±0.64b	73.80±0.54b	76.50±0.48b
11.5	72.20±0.46a	79.98±0.50a	43.17±0.53a	47.88±0.49a	46.55±0.47a	53.89±0.49a	258.54±0.56a	242.28±0.59a	76.13±0.66a	81.74±0.55a

Values are in mean±standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$) through Duncan's test

MCKPI-N natural Meghalayan cherry kernel protein isolate, *MCKPI-DP* dephenolized Meghalayan cherry kernel protein isolate

in pH is due to a change in hydrophilic-lipophilic balance directly connected to emulsion properties [34]. Additionally, increased pH also increases the columbic interactions between neighboring droplets coupled with hydration of charged molecules, thus increasing the emulsifying properties [8]. The emulsion activity was comparable with sunflower kernel protein isolate (48.86–53.35%) [14] and sour cherry kernel protein isolate [35] whereas lower emulsion activity was reported as compared to Chenopodium album protein isolate (55.09–64.70%) [8].

MCKPI-DP showed higher emulsion activity and emulsion stability as compared to MCKPI-N. This is because of a decrease in solubility and hydrophobicity of protein isolate due to phenolics. As increased solubility and hydrophobicity is the precondition for a good emulsifier. The decreased solubility blocks the fast movement of peptides to the oil–water interface. The reduction in the surface hydrophobicity of protein isolate may decrease the capacity of protein to localize at the oil–water interface. Phenolic compounds also enhance protein aggregation, which reduced the hydrophobicity and thus reduce the unfolding at the interface [14].

Water-binding capacity Table 3 shows the effect of dephenolization and pH on the water holding capacity of cherry kernel protein isolate. The protein isolate (MCKPI-DP) formed after phenolic removal showed lower WBC compared to protein isolate (MCKPI-N) with phenolics. This occurred at all the pH values. It might be because of the reduction in hydrophobicity of protein upon interaction with phenolics. Also, the phenolics present in the protein isolate have many binding sites for water, where it gets through hydrogen bonding and enhances the water binding capacity [14].

The pH had a significant effect on the WBC of protein isolate in both cases MCKPI-N and MCKPI-DP. With the increase in pH, the WBC increases from 202.86±0.80 to 242.28±0.59% for MCKPI-DP and 214.65±0.59 to 258.54±0.56% for MCKPI-N. Similar outcomes were acquired by Mir et al. [8] and Malik and Saini [14]. At isoelectric pH, the lowest WBC was seen in both cases because of strong intermolecular interactions to form aggregates, leading to less interaction and less WBC [36]. The different WBC at different pH might be because of conformational characteristics in protein structure, which influences the binding sites for water. Cherry kernel protein isolate showed higher WBC than pea and soy protein isolate [37], chenopodium album [8], and sunflower meal [14] protein isolate. Higher WBC of cherry kernel protein isolate proposes its appropriateness for enhancing the viscosity of food products.

Dispersibility Dispersibility is the reconstitution capacity of proteins and is mainly affected by the temperature, ionic composition, pH, and the degree of agitation of solvent. Increased value of dispersibility amplifies other functional

characteristics like emulsifying and foaming ability. A significant increase in dispersibility was seen with the increase in pH from 3.5 to 11.5 for MCKPI-N and MCKPI-DP. The dispersibility value for MCKPI-N and MCKPI-DP varied from 65.44 ± 0.80 to $81.13 \pm 0.66\%$ and 66.83 ± 0.63 to $86.74 \pm 0.55\%$, respectively, with the highest value was reported at pH 11.5. As solubility is directly connected to dispersibility, it increased with increased pH as seen in the case of solubility. Similarly, dispersibility increases with phenol removal because solubility increases with the removal of phenols.

Foam capacity and stability Foam formation and characteristics are affected by the type of protein, preparation procedure, composition, solubility, concentration, pH, salts present, and hydrophobic interactions. Foaming capability depends on the diffusion of soluble proteins to the air–water interface, faster conformational modifications and rearrangement at the interface [27]. Proteins with more foaming capability and foam stability are needed in many food systems and are mainly used in food applications for aeration and whipping. A significant difference was seen between foaming capacity and foam stability in the case of both MCKPI-N and MCKPI-DP (Table 4). With the increase in pH the foam capacity increased significantly, and the highest value was obtained at pH 11.5 for both MCKPI-N and MCKPI-DP, i.e., $51.33 \pm 0.36\%$ and $57.80 \pm 0.53\%$, respectively. Higher foaming capacity was observed in MCKPI as compared to sour cherry kernel protein isolate ($35 \pm 3.4\%$) [35], and sunflower kernel protein isolate ($57.31 \pm 0.50\%$) [14]. Whereas similar increase in foam capacity with pH was seen by Das et al. [38] and Mir et al. [8]. Aluko and Yada [39] showed that at increased pH values, the total charge on proteins is raised, which effects in weakening of hydrophobic interactivity but enhances the flexibility of proteins. Higher protein flexibility helps protein diffuse rapidly to the air–water interface to encapsulate air, resulting in increased foam formation. The lowest foam capacity and stability after 60 min were found at pH 5.5 due to protein behavior at isoelectric pH. Damodaran [27] reported that only soluble protein fractions are involved in the foam formation and since near the isoelectric point, the concentration of these soluble fractions are very low and thus amount of foam formation will be less. Similarly, like foam capacity, the foam stability also increased with an increased pH, but as time progresses stability of foam decreases significantly for both MCKPI-N and MCKPI-DP. Similar results were obtained for sour cherry kernel, sunflower seed protein isolate [17, 35].

The presence of polyphenols directly affects the foaming capability and stability due to impaired solubility and hydrophobicity of proteins which decreases its foaming properties,

Table 4 Effect of dephenolization and pH on foaming properties of Meghalayan cherry kernel protein isolate

pH	Foam capacity (%)		Foam stability (%)							
			15 min		30 min		45 min		60 min	
	MCKPI-N	MCKPI-DP	MCKPI-N	MCKPI-DP	MCKPI-N	MCKPI-DP	MCKPI-N	MCKPI-DP	MCKPI-N	MCKPI-DP
3.5	34.10±0.32d	37.20±0.77d	65.30±0.37d	68.03±0.41d	62.83±0.57d	65.10±0.43d	55.73±0.61d	59.60±0.37d	48.26±0.44d	52.86±0.70d
5.5	29.83±0.41e	31.83±0.44e	60.66±0.38e	62.53±0.49e	57.10±0.65e	59.83±0.41e	52.60±0.50e	55.73±0.53e	45.70±0.48e	48.66±0.49e
7.5	37.96±0.46c	41.43±0.65c	68.83±0.61c	72.06±0.54c	66.90±0.57c	69.70±0.53c	58.76±0.53c	63.10±0.49c	51.93±0.61c	58.80±0.37c
9.5	44.23±0.33b	48.06±0.49b	72.70±0.53b	75.86±0.46b	69.03±0.63b	73.63±0.57b	62.10±0.49b	69.93±0.36b	55.63±0.54b	64.13±0.36b
11.5	51.33±0.36a	57.80±0.53a	74.93±0.65a	79.10±0.24a	70.96±0.62a	76.06±0.44a	64.03±0.57a	71.96±0.49a	58.90±0.53a	66.93±0.53a

Values are in mean ± standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$) through Duncan's test

MCKPI-N natural Meghalayan cherry kernel protein isolate, *MCKPI-DP* dephenolized Meghalayan cherry kernel protein isolate

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Table 5 Amino acid composition of MCKPI-N and MCKPI-DP with respect to provisional scoring pattern of FAO (2013)

Amino acid (g/kg)	MCKPI-N	MCKPI-DP	FAO (2013) (infants)	FAO (2013) (adults)
Aspartic acid	45.86 ± 0.40b	49.83 ± 0.52 a		
Glutamic acid	141.76 ± 0.64b	151.53 ± 0.91a		
Serine	54.53 ± 0.41	42.6 ± 0.37b		
Histidine	31.46 ± 0.49b	36.56 ± 0.61a	20	16
Glycine	91.06 ± 0.62b	98.66 ± 0.59a		
Threonine	39.96 ± 0.65a	38.06 ± 0.46b	31	25
Arginine	41.46 ± 0.49b	50.06 ± 0.62a		
Alanine	65.16 ± 0.57b	71.23 ± 0.77a		
Tyrosine	39.93 ± 0.60a	40.1 ± 0.69a		
Cysteine	5.43 ± 0.12a	5.6 ± 0.28a		
Valine	81.86 ± 0.36b	89.56 ± 0.57a	43	40
Methionine	21.93 ± 0.38b	26.53 ± 0.65a		
Phenylalanine	16.53 ± 0.32b	18.76 ± 0.73a		
Isoleucine	29.96 ± 0.44a	25.63 ± 0.47b	32	30
Leucine	71.03 ± 0.36b	74.63 ± 0.6a	66	61
Lysine	58.83 ± 0.41b	68.73 ± 0.65a	57	48
Proline	6.23 ± 0.24b	9.76 ± 0.33a		
ΣSAA[1]	27.37 ± 0.49b	32.13 + 0.73a	27	23
ΣAAA[2]	56.46 ± 0.46b	58.86 ± 0.61a	52	41
ΣEAA[3]	351.86 ± 0.76b	378.56 ± 0.61a		
ΣNEAA[4]	519.33 ± 0.95a	491.46 ± 0.69b		
ΣNAA[5]	354.13 ± 0.63a	322.53 ± 0.96b		
ΣHAA[6]	94.50 ± 0.45a	80.66 ± 0.40b		
ΣAcAA[7]	187.63 ± 0.67b	201.36 ± 0.38a		
ΣBAA[8]	125.36 ± 0.49b	155.36 ± 0.65a		
ΣTAA[9]	842.3 ± 0.54b	897.9 ± 0.94a		

Values are in mean ± standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$) through Duncan's test

MCKPI-N natural Meghalayan cherry kernel protein isolate, *MCKPI-DP* dephenolized Meghalayan cherry kernel protein isolate

[1]Sulfur amino acid: Cys + Met

[2]Aromatic amino acid: Phe + Tyr

[3]Essential amino acid: Leu + Ile + Met + Cys + Phe + Tyr + Val + His + Lys + Thr + Trp

[4]Nonessential amino acid: Asp + Glu + Ser + Pro + Gly + Ala + Arg

[5]Neutral amino acid: Ala + Ser + Leu + Ile + Gly + Pro

[6]Hydroxylic amino acid: Ser + Thr

[7]Acidic amino acid: Asp + Glu

[8]Basic amino acid: Lys + His + Arg

[9]Total amino acid

and thus, natural protein isolate has lower foam capacity compared to dephenolized protein isolate.

3.3 Amino acid profiling of protein isolates

Table 5 present the amino acids profile of protein isolates from natural and dephenolized Meghayalan cherry kernel. Removing phenolics caused significant differences in amino acids composition in MCKPI-N and MCKPI-DP. This might be due to polyphenols (mainly phenolic acids and flavonoids) blocking amino acid residues and decreasing their availability. For example, phenolic compounds in alkaline conditions react with oxygen to form phenolate ions, leading to the formation of semiquinones. These semiquinones react with an amino group of lysine and decrease their availability. Similarly, the indole structure of tryptophan is involved in the reaction with polyphenols [40].

Results showed that both MCKPI-N and MCKPI-DP are a rich sources of essential amino acids where sulfur-containing amino acids like methionine and cysteine are low in an amount, which may be because of solubility of albumins in water and sulfur-containing amino acids are partially removed due to washing of kernel powder before alkaline treatment [41]. Higher essential amino acids were found in the case of MCKPI-DP. Furthermore, higher content of both aspartic and glutamic acid (i.e., 38.9 and 49.8 g/kg and 172.1 and 181.5 g/kg) was found in MCKPI-N and MCKPI-DP, respectively. Higher content of these amino acids indicates the acidic properties of both protein isolates. Leucine, valine, and isoleucine are considered as essential branched amino acids and are required for building the muscles and both MCKPI-N and MCKPI-DP exhibited higher content of all these amino acids. The leucine, valine, and isoleucine content in MCKPI-N and MCKPI-DP was 74.6, 89.6, and 25.6 g/kg and 71, 81.9, and 30.1 g/kg, respectively. Some of the amino acids present in Meghayalan cherry kernel protein isolate like tyrosine, methionine, histidine, and lysine have been shown to play a specific role in improving the antioxidant property of peptides [42]. The threonine content in MCKPI-N and MCKPI-DP was 38.1 and 40.1 g/kg, respectively, which helps maintain the positive nitrogen balance in body as it helps in lowering nitrogen excretion [43].

3.4 Nutritional quality of protein isolates

The nutritional profile of protein isolate based on the composition of amino acids is presented in Table 6. The protein quality is assessed as per the basis of its amino acid composition as compared to reference pattern of amino acids. The nutritional quality of protein is measured as per the basis of amino acid score, protein efficiency ratio (PER), biological value, nutritional index, essential amino acid index, and ratio of essential amino acid to total amino acid (E/T %). The theoretical protein efficiency ratio that bears a good relationship to real PER values was calculated depending upon the concentration of leucine and proline (PER_1), leucine and tyrosine (PER_2), and methionine, leucine, histidine, and tyrosine (PER_3). Generally, a PER value less than 1.5 approximately shows lower protein quality; a value between 1.5 and 2.0 as medium quality, while a value more than 2.0 is of good quality [44]. The Meghayalan cherry protein isolate has a PER value higher than 2 for both MCKPI-N and MCKPI-DP and is considered high-quality protein. The present result showed higher PER value in Meghayalan cherry protein isolate than cowpea (1.21), pigeon pea (1.82), and peanut (1.45–1.76) [45]. However the results were comparable with pumpkin seed protein isolate (2.70) [46]. Protein efficiency ratio is directly connected to the leucine contents; therefore, any change in the leucine content will affect the protein efficiency ratio. The percentage of E/T for MCKPI-N and MCKPI-DP was seen to be $37.63 \pm 0.01\%$ and $41.76 \pm 0.01\%$, respectively, which is almost similar to lotus and pumpkin seed proteins as well as soya protein [46, 47]. Higher E/T % was found in dephenolized Meghalayan cherry kernel protein isolate because of the increased amount of essential amino acid present (Table 6).

Biological value (BV) is the percentage of the protein which is used as the proteins of the human body. The biological value of better nutritional quality protein foods is found to be in between 70 and 100% [18]. The biological value has given a measurement of the body's efficiency to utilize the protein consumed in the diet. A food with more BV value connected to the good supply of EAAs [48]. Our study showed that dephenolized protein isolate have a higher biological value (74.22 ± 0.93) than natural protein isolate (66.06 ± 0.84) and thus can be incorporated in efficient protein diets. The nutritional index is the function of essential amino index and protein content of the sample. It is known that higher the protein content in the sample more will be the nutritional index. The nutritional index of MCKPI-N and MCKPI-DP was 63.35 and 65.82, respectively.

Egg was the first to be assigned a chemical score as it was considered nutritionally complete [49]. The EAAI may be defined as the geometric mean value of EAA content in a protein compared with a corresponding amount in the reference whole egg protein. The protein quality rating was observed when all of the individual chemical scores were used to calculate EAA (Table 5). For the essential amino acid index, it has been observed that protein-based food products are of good quality when their essential amino acid index is greater than 90 and are considered useful when their essential amino acid index ranges between 70 and 80. Also, the nutritional quality of a food is entitled as insufficient when its essential amino acid index is below 70 [34]. Both natural and dephenolized protein isolate of Meghalayan cherry kernel have EAAI higher than 70, i.e., 71.34 ± 0.77 and 78.82 ± 0.85, respectively, and are considered as useful protein isolates. Higher EAAI was found dephenolized protein isolate due to higher essential amino acids.

The branched-chain amino acids (BCAA) (e.g., valine, leucine, and isoleucine) are involved in promoting the anabolic processes in protein metabolism, helpful in preventing proteins from degradation, associated with protein synthesis and also promoting muscle growth after physical activity [50]. An increase in the leucine content improves protein synthesis and or retardation in muscle degradation. Therefore, diets containing more leucine to isoleucine ratio can provide more endurance than natural diets. Higher leucine to isoleucine ratio was observed in dephenolized protein isolate (2.91 ± 0.03) as compared to natural protein isolate (2.37 ± 0.02).

Table 6 Nutritional profile of protein isolate from MCKPI-N and MCKPI-DP

Parameter	MCKPI-N	MCKPI-DP
PER$_1$	2.26 ± 0.03b	2.67 ± 0.03a
PER$_2$	2.32 ± 0.01a	2.49 ± 0.02a
PER$_3$	1.48 ± 0.03b	2.14 ± 0.04a
E/T (%)	37.63 ± 0.01b	41.76 ± 0.01a
BV	66.06 ± 0.84b	74.22 ± 0.93a
Nutritional index	63.35 ± 0.66b	65.82 ± 0.71a
EAAI	71.34 ± 0.77b	78.82 ± 0.85a
Leucine/isoleucine ratio	2.37 ± 0.02b	2.91 ± 0.03a

Values are in mean ± standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$) through Duncan's test
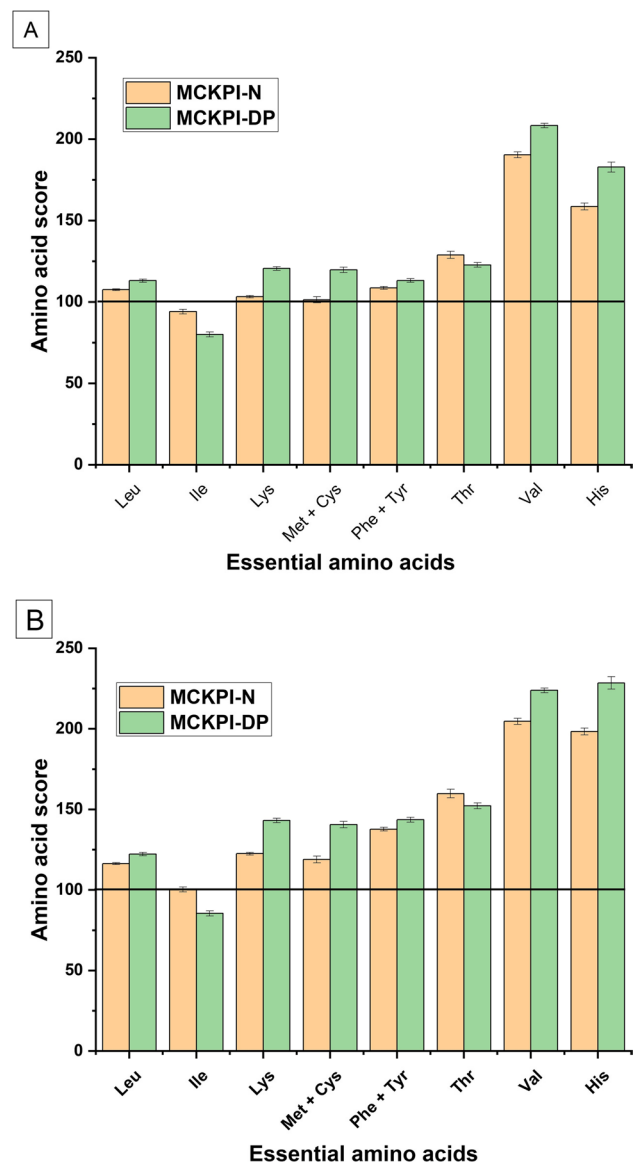
MCKPI-N natural Meghalayan cherry kernel protein isolate, *MCKPI-DP* dephenolized Meghalayan cherry kernel protein isolate, *PER* protein efficiency ratio, *E/T* essential amino acid/total amino acid, *BV* biological value, *EAAI* essential amino acid index

3.5 Amino acid score for protein quality evaluation

The protein quality is based on its capacity to provide an appropriate patterns of dietary indispensable amino acids [20]. The amino acid score was computed based on its composition to know the limiting amino acid. The amino acid score for infants and adults was shown in Fig. 1. The corresponding amino acid is considered as limiting amino acid if its amino acid score is less than 100. Based on the amino acid score, isoleucine has been reported as limiting amino acid for both MCKPI-N and MCKPI-DP in infants, whereas for adults, only MCKPI-DP showed isoleucine as the limiting amino acid. The MCKPI-N and MCKPI-DP presented higher protein quality than commercial protein supplements from pea protein isolate as methionine + cysteine, which are sulfur containing amino acids with powerful antioxidant properties, present in higher amounts in Meghalayan cherry protein isolate as compared to legumes which lack sulfur-containing amino acids. These results showed that Meghalayan cherry protein isolate can be utilized as a protein supplement source.

4 Conclusion

The significant effect of dephenolization was seen in the functional, physicochemical and nutritional properties of Meghayalan cherry kernel protein. Increased pH increases the yield of protein isolate, but the purity (protein content) of protein isolate decreased significantly. The color of protein isolate was also improved after the removal of phenolics from kernel powder. The significant increase in functional properties for both MCKPI-N and MCKPI-DP was seen with increase in pH. However, MCKPI-DP showed improved

Fig. 1 Amino acid scoring with respect to amino acid scoring pattern of FAO for infants (**A**) and adults (**B**) of natural (MCKPI-N) and dephenolized (MCKPI-DP) Meghalayan cherry kernel protein isolate

functional properties as compared to MCKPI-N. In terms of amino acid composition, the significant difference was observed between MCKPI-N and MCKPI-DP with a higher value of essential amino acids found in the case of MCKPI-DP. However, both MCKPI-N and MCKPI-DP showed good amount of essential amino acids but based on amino acid score, both the protein isolate were limiting in isoleucine. The nutritional values (PER, EAAI, BV, and nutritional index) of MCKPI-DP were higher than MCKPI-N and are considered as good quality useful proteins. On the basis of amino acid score for adult and infants, isoleucine was found to be limiting amino acid for both MCKPI-N and MCKPI-DP. These finding showed that dephenolized Meghayalan

cherry kernel proteins isolate has improved functional properties with good balance of essential amino acids. Thus, it could be utilized as a novel protein source in food systems as well as to formulate various health foods. In the future, the effect on dephenolization on in vitro protein digestibility, thermal and structural properties of MCKPI need to be explored in order to better utilize it in various food products.

Declarations

Conflict of interest The authors declare no competing interests.

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