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PROTEIN CONCENTRATE EXTRACTED FROM GRAPE SEEDS: IMPACT OF DIFFERENT PH LEVELS ON THE AMINO ACID COMPOSITION, STRUCTURAL, THERMAL, MORPHOLOGICAL, AND FUNCTIONAL PROPERTIES

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ABSTRACT

Background. Grape seeds, a major by-product of wineries, contain a high amount of protein and could potentially be exploited as a promising source of protein concentrate. There is a growing need to extract protein from vegetable sources, as opposed to animal sources, due to growing environmental concerns.
Materials and methods. The protein concentrate was prepared from defatted grape seed meal by modifying the extraction pH at four levels i.e. 8, 9, 10, and 11. The effect of different pH levels on the amino acid composition and its structural, thermal, morphological and functional properties were studied.
Results. The extracted grape seed protein concentrate resulted in a maximum yield and purity value of 12.23 ±0.23% and 55.66 ±0.15%, respectively, at different pH values. With an increase in the pH of grape seed protein concentrate, an increase in solubility, foaming stability and capacity, emulsifying capacity, and water and oil holding capacity were observed. In the flow properties of grape seed protein concentrate extracted at different pH levels, significant changes were observed in the loose bulk density, packed bulk density, true density, Hausner's Ratio, Carr's Index, and angle of repose of the protein concentrate. The SEM analysis revealed that protein in the pH-adjusted samples showed a more condensed and clustered shape. The grape seed protein concentrate extracted at pH 10 exhibited greater thermal stability, with a denaturation temperature of 104.23 ±2°C, in comparison to samples extracted at other pH levels.
Conclusions. Adjusting the pH during the extraction process can be used as a promising technique to enhance the quality of grape seed protein concentrate, and has a variety of applications in various food formulations.

Keywords: grape seed, protein concentrate, amino acid, thermal property, functional properties

INTRODUCTION

Given the ongoing growth of the global population, there is a rising need for sustainable as well as substitute protein sources to fulfil the nutritional requirements of the masses. Protein, as a macronutrient, is crucial for sustaining bodily development as well as promoting growth rate. The worldwide need for protein is projected to increase by 200% by the year 2050. Industries, along with other research communities, are increasingly interested in exploring plant protein sources, instead of animal proteins, due to several factors. These include limited supply, rising market prices, environmental impacts like carbon footprint and greenhouse gas emissions, moral and religious convictions of consumers, and the prevalence of animal

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diseases like avian influenza (Bird flu) and swine influenza (Swine flu) (Kumar et al., 2022). Plant proteins are currently being used to generate meat substitutes that closely resemble animal meat in terms of taste, appearance, mouth feel, and texture. Furthermore, it is critical to identify sustainable plant protein sources that are not fully explored in order to alleviate the economic strain on prominent soy, pea, or other proteins, and decrease the reliance on animal protein.

Oilseeds are plants that are mainly grown for the purpose of extracting oil from their seeds. They also contain many other biological macromolecules. They have a crucial impact on worldwide agriculture and the food sector, providing diverse economic and nutritional advantages. Oilseed proteins have the potential to meet these requirements. In 2018, 79 million tonnes of harvested grapes were processed, accounting for almost 75% of the global total (FAOSTAT, 2021). The primary byproduct of wineries is grape pomace, also known as marc, which amounts to about 10 million tonnes annually and consists primarily of grape seeds as well as skins. As far as the composition of grape seed powder is concerned, it contains 8.74% moisture content, 13.67% crude protein content, 2.89% ash content and 19.47% oil content. The oil content of grape seed is around 20% (w/w), resulting in the production of defatted grape seed meal (DGSM) as a by-product. DGSM is often regarded as a highly abundant and valuable reservoir of organic material. However, to the present day, this residue of grape seeds is primarily discarded as biomass. This waste has the potential to be a valuable source of vegetable protein, peptides, or amino acids due to its large amount of available feedstock. As far as protein extraction is concerned, from these agro-industrial wastes, protein can be obtained from plants by standard extraction methods, such as employing solvents or alkaline-based techniques. The extraction process is dependent on the composition and quality of the protein in the raw material, and has a significant impact on the yield, composition, and techno-functional aspects of the resulting extracts. The protein's composition and quality are mostly dictated by its previous treatment. Presently, the most economically efficient method for producing vegetable protein ingredients on a large scale is alkaline extraction followed by acid precipitation. The pH used during extraction can impact the interactions between protein molecules and their structural orientation, ultimately affecting the nutritional content (Song et al., 2023). So far, multiple experiments have been carried out to extract plant protein from sources such as mung bean, sunflower seed, soybeans, and buckwheat (Liu et al., 2021). Baca-Bocanegra et al. (2021) conducted a study on extracting protein from defatted grape seed flour. In India, agro-industrial waste disposal is a major concern as it leads to health hazards, environmental degradation as well as economic losses caused by inadequate management of excessive waste. Huge tonnes of grape seeds are discarded by wineries as a waste material. There is a need to find ways to mitigate these issues. Grape seeds, used for the production of protein, is a step towards a circular economy. For the first time, grape seed protein concentrate was prepared from Indian grapes (Bangalore blue variety). The previous researchers optimised the process variables to achieve the highest level of protein purity but did not perform thorough characterization. Therefore, comprehensive investigations into the preparation and characterization of grape seed protein concentrate, across various levels of extraction pH, are scarce. The objective of this study was to study the nutritional features, functional properties, and intermolecular changes of grape seed protein concentrate (GSPC) extracted at different pH levels.

MATERIALS AND METHODS

Raw materials and chemicals

The grape seeds were procured from Elite Vintage Winery in Karnataka, India. The seeds were manually cleaned to remove any undesirable materials. Sigma Aldrich chemicals were purchased in Chandigarh, India. The analysis was conducted using Millipore-filtered water (Merck, Progard TS2, PR0G0T0S2, India).

Preparation of grape seed protein concentrate (GSPC)

The grape seeds were rinsed with water and placed in a tray drier at a temperature of 40°C overnight. The grape seeds were then pulverised to obtain a grape seed flour. Hexane was employed to extract defatted grape seed meal by utilising the soxhlet apparatus (Soxhlet Extractor 431/8, JSGW; Heating mantle-1522/6, The

Laboratory Glassware Co., India). The preparation of grape seed protein concentrate (GSPC) was done according to the methodology outlined by Kornet et al. (2022), with minor adjustments. During protein extraction, the defatted grape seed meal was diluted with distilled water in a ratio of 1:10. The pH levels (8, 9, 10, and 11) were then adjusted by adding 1N NaOH. The solutions were agitated for a duration of 3 h to enhance the pace at which the protein dissolves, and then subjected to centrifugation with a force of 6000 g for 30 min, at a temperature of 4°C, in order to gather the liquid portion above the sediment. The suspension with a high concentration of protein was subsequently subjected to filtration in order to eliminate the fractions that were not dissolved. The supernatant pH was lowered to reach its isoelectric point (pH 3.2) by adding 1 N HCl, while stirring continuously for 30 min. The mixture was then centrifuged at 6000 g for 30 min at 4°C until separation occurred, withthe precipitated proteinbeing then collected. The grape seed protein concentrate (GSPC) was subsequently freeze-dried (Typ 101021, Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The dehydrated grape seed protein concentrate was converted into a fine powder and stored in plastic pouches for subsequent analysis.

Characterization of GSPC

Yield, purity, moisture and fat

The protein purity and yield of GSPC samples were determined as stated by Mir et al. (2019). The protein content of GSPC samples was estimated using the Kjeldahl method (N×5.75). The moisture content and fat content of GSPC samples were determined based on the methodology described by Gao et al. (2020). The grape seed protein yield was determined using the following equation.

$$\text{Extraction Yield (\%)} = \text{concentrate weight (g)} / \text{Flour weight (g)} \times 100$$

$$\text{Purity (\%)} = \text{total protein in concentrate (\%)} \times \text{weight of concentrate (g)} / \text{weight of concentrate (g)}$$

$$\text{Fat content (\%)} = (B - C) / A \times 100$$

where

A = sample weight

B = weight of flask after extraction

C = weight of flask prior to extraction.

Loose bulk density, packed bulk density and flow properties

The loose bulk density (ρB) and packed bulk density (ρT) were measured using a 25 mL measuring cylinder, following the method given by Chinta et al. (2009). Approximately 3 g of the sample (Mo) was carefully poured via a funnel into the cylinder. Then, the cylinder was gently tapped to gather the powder adhering to the inner surface of the cylinder. The volume (Vo) was directly measured from the cylinder to compute the loose bulk density (ρB = Mo/Vo). To get the packed bulk density (ρT = Mo/Vc), tapping was performed 100 times for each sample until a consistent volume (Vc) was achieved. The flowability of GSPC powder was assessed by the Carr's Index (CI) and the Hausner Ratio (HR). The CI and HR were computed by employing the respective equations.

$$CI = 100 \times (\rho T - \rho B)/(\rho T),\ HR = \rho T/\rho B.$$

Wettability and water activity (a_w)

The wettability of GSPC was assessed using the method developed by Freudig et al. (1999) with slight modifications. A blocked funnel was positioned on a ring stand above a beaker containing 50 mL of water. Subsequently, 0.5 grams of GSPC powder was put into the funnel, while the block was removed to allow the sample to transfer into the beaker. The duration of GSPC sample penetration was measured using a stopwatch. For measuring water activity, GSPC samples (1.5 g) were put in the measuring cell of the water activity metre (Rotronic, Hygrolab, Bassersdorf, Schweiz, Switzerland), and a_w was measured at room temperature.

Functional properties

Protein solubility (PS) was quantified using the methodology outlined by Gao et al. (2020). PS was calculated by using the following equation:

$$\text{PS (\%)} = \text{Protein content in supernatant} / \text{Protein content of the sample} \times 100\%$$

The water-holding-capacity (WHC), oil-holding-capacity (OHC), foaming capacity (FC), and

emulsifying capacity (EC) of GSPC were estimated using the methodology described by Deb et al. (2022). WHC/OHC was calculated using the following equation:

WHC or OHC (g/g) = Sample weight after centrifugation – Initial sample weight/ Initial sample weight

FC of GSPC samples was determined using the following equation:

$$FC\ (mL) = V_2 / V_1$$

where

V_1 is the volume of protein solution (mL)

V_2 is the volume of foam (mL).

EC of GSPC samples was determined using the method described by Lawal et al. (2007).

$$EC\ (\%) = H_e / H_T \times 100$$

where

H_e is the height of emulsion

H_T is the total height.

Sulfhydryl (SH) group and disulfide bond (SS) contents

GSPC samples were analysed to determine the amount of sulfhydryl groups (SH), as well as disulfide bonds (SS), using the method described by Gao et al. (2020). The SH content of GSPC was quantified using the following equation and presented in units of μmol/g. The measurement of absorbance was conducted at a wavelength of 412 nm.

$$SH = 73.53 \times A \times D/C$$

where

A is the absorbance

C is the concentration of the sample

D is the dilution factor.

Similarly, the SS content was determined using the equation:

$$SS\ (\mu mol/g) = Total\ SH - free\ SH/2$$

Colour

The colour of GSPC samples was assessed according to the method outlined by Sheikh et al. (2022), using a Hunter colorimeter (Colour i5, GretagMacbeth, CH-8105 Regensdorf, Switzerland) within the CIE L^*, a^*, b^* colour region. The aforementioned values were subsequently utilised to compute the changes in overall colour variance (ΔE*), chroma, and hue angle of GSPC, employing the corresponding formulae outlined below:

$$\Delta E^* = \sqrt{[L^* - Lo]^2 + [a^* - ao] + [b^* - bo]^2}$$

where

Lo, *bo* and *ao* indicate the colorimetric parameters of GSPC.

Amino acid and nutritional parameters

The amino acid composition of GSPC was determined using the method described by Zumwalt et al. (1987), with minor adjustments made. 100 mg of GSPC sample was placed in a test tube, and amino-butyric acid (0.5 M) was added at a volume of 10 μL, followed by the addition of HCl (6 N) at a volume of 5 mL. Subsequently, the resulting GSPC samples were subjected to cooling in order to facilitate HPLC analysis using a C18 column of 150 × 4.6 mm (Agilent 1100, Germany). The detection of primary amino acids was performed using a fluorescence detector that had an excitation wavelength of 335 nm and an emission wavelength of 440 nm. O-phthaldialdehyde was used as a derivative reagent for this purpose. The detector was configured to 260 nm (excitation) and it detected 315 nm (emission) at 22 min. This was done by employing 9-fluorenylmethyl chloroformate for determining secondary amino acids. Amino acids were quantified using the calibration curves with different amino acid standards (A9781, Sigma Aldrich). Additionally, alkaline hydrolysis using a 5 M NaOH solution was conducted to determine the amount of tryptophan present. The nutritional properties of GSPC samples were calculated using the following formulae:

Protein efficiency ratio (PER) (g/100 g) were calculated as follows:

$$PER\ 1 = -0.684 + 0.456(Leu) - 0.047(Pro)$$

$$PER\ 2 = -0.468 + 0.454(Leu) - 0.105(Tyr)$$

Differential scanning calorimetry (DSC)

The thermal properties of GSPC samples were analysed using a differential scanning calorimeter (PerkinElmer,

DSC 4000, N520-0112, USA), following the procedure described by Salgado et al. (2011).

FTIR

The Fourier Transform Infrared Spectrums (FTIR) of GSPC samples were obtained using an FTIR spectrophotometer (PerkinElmer, Spectrum Two FT-IR Spectrometer, L160000A, New Delhi, India). The sample was subjected to scanning from 600 to 4000 cm^{-1}, at a resolution equal to 2 cm^{-1} at 32 scans. The changesin the secondary structure of GSPC were assessed by analysing the overlapping components in the 1700 to 1600 cm^{-1} range, namely the broad amide-I band, using the methodology described by Sofi et al. (2021).

Morphological analysis

The morphological properties of GSPC samples were examined by field emission scanning electron microscopy (JEOL JSM 7610 F PLUS, Musashino, Tokyo, Japan), using the approach described by Mir et al. (2019). Prior to examination, the GSPC samples were subjected to gold particle coating using a sputter coater.

Statistical analysis

The data analysis was conducted using IBM's statistical tool, SPSS Statistics (Version 26). Duncan's multiple range test (DMRT) was used to assess significant variations among the average values. The level of significance was assessed at 95% confidence level.

RESULTS AND DISCUSSION

Yield, purity, moisture, and fat content

The pH used for extraction had a substantial impact ($p < 0.05$) on both the yield as well as the chemical composition of GSPC (Table 1). The highest GSPC output was observed at a pH of 11 (12.23%). The elevation of the pH during extraction likely led to the disruption of hydrogen bonds present between the carbolic and sulphate groups across the cell membrane, hence enhancing protein yield. Furthermore, increasing the pH during the extraction process improved the solubility of proteins, increased the concentration of storage and structural proteins, increased the ionisation of carboxylic groups and the number of deprotonated amine groups. Therefore, increasing the extraction pH had a significant effect on increasing the yield of protein extracted. In a comparable manner, Baca-Bocanegra et al. (2021) carried out a process optimisation procedure for the extraction of grape seed protein, in the pH range from 8.5 to 10.5, and found that pH 10 showed maximum protein content. Gao et al. (2020) found that raising the extraction pH from 8.5 to 9.5 resulted in an increased yield of pea protein extracted, increasing the percentage from 12.93 to 15.36. Arogundade et al. (2006) found that increasing the pH of the extraction process from 7 to 12 pH resulted in a higher protein extraction yield of broad bean concentrate, with an increase from 25.80% to 32.40%.

However, in the case of GSPC, increasing the extraction pH from 8 to 11 resulted in an increase in the extraction yield from 3.64% to 12.23%. The protein purity increased as the pH shifted from 8 to 9, and subsequently decreased as there was a further increase in pH from 10 to 11, potentially leading to an increase in the availability of carbs and other components. The extraction of protein at pH 9 showed the highest purity in the GSPC samples, measuring at 55.66%. In addition, the GSPC samples showed moisture content ranging from 2.01 to 2.05%. There were no statistically significant variations ($p < 0.05$) in the moisture and fat levels of the GSPC samples. The current results are consistent with the findings of Arogundade et al. (2006) in the broad bean protein concentrate.

Loose bulk density, packed bulk density, and flow properties

GSPC exhibited a loose bulk density ranging from 0.833 to 0.721 (g/mL) throughout the pH levels of 8 to 11 (Table 1). GSPC powder achieved the highest packed bulk density of 0.970 g/mL at pH 8, whereas at pH 11, it exhibited the lowest packed bulk density of 0.819 g/mL. The decrease in loose bulk density may be attributed to the solidification of the bigger particles. Protein concentrates with lower viscosity prior to freeze drying were shown to yield products that had lower bulk density. Carr's index (CI), as well as Hausner's ratio (HR), are crucial factors to be taken into account when dealing with the reconstitution, packaging, and transportation of microencapsulated powders. Greater values of these parameters indicate that the powder has a higher level of cohesion and, as a result, does not flow freely. GSPC powder's Carr's

Table 1. Flow properties, foaming capacity, stability, chemical compositions, total sulfhydryl groups, free sulfhydryl groups of grape seed protein concentrate (GSPC) extracted at different pH

Parameters	Treatments			
	pH 8	pH 9	pH 10	pH 11
Loose bulk density, g/ mL	0.833 ±0.02[a]	0.769 ±0.03[b]	0.735 ±0.01[c]	0.721 ±0.01[d]
Packed bulk density, g/ mL	0.970 ±0.04[a]	0.909 ±0.01[b]	0.852 ±0.03[c]	0.819 ±0.02[d]
True density, g/ mL	0.953 ±0.05[a]	0.867 ±0.09[b]	0.819 ±0.08[c]	0.781 ±0.11[d]
Porosity, %	12.70 ±0.37[a]	11.32 ±0.45[b]	10.29 ±0.91[c]	7.70 ±0.78[c]
Hausner's Ratio	1.11 ±0.07[c]	1.18 ±0.02 [a]	1.10 ±0.080[d]	1.13 ±0.01[b]
Carr's Index, % of compressibility	14.12 ±0.98[c]	15.39 ±0.68[a]	13.76 ±0.59[c]	11.94 ±75[d]
Angle of repose, ◦	0.72 ±0.01[c]	0.70 ±0.02[d]	0.82 ±0.02[b]	1.04 ±0.03[a]
Wettability, min	14.03 ±1.0 [a]	12.01 ±0.3 [b]	11.33 ±0.5 [c]	09.11 ±0.9 [d]
Water activity, a_w	0.48 ±0.0019 [d]	0.49 ±0.0017 [c]	0.50 ±0.0025 [b]	0.51 ±0.0031 [a]
Foaming capacity, mL	2.2 ±0.1 [d]	2.9 ±0.2 [c]	5.8 ±0.4 [b]	8.0 ±0.3 [a]
Foaming stability				
15 min	102.04 [b]	102.02 [b]	102.86 [b]	105.88 [a]
30 min	101.71 [d]	101.97 [c]	102.70 [b]	103.84 [a]
45 min	101.01 [d]	101.93 [c]	102.58 [b]	101.71 [a]
Extraction yield, %	3.64 ±0.34[d]	6.56 ±0.67 [c]	8.62 ±0.81[b]	12.23 ±0.23[a]
Purity, %	52.4 ±0.53 [b]	55.66 ±0.15 [a]	51.12 ±0.79 [c]	48.45 ±0.45 [d]
Moisture, %	2.03 ±0.01[a]	2.01 ±0.02 [a]	2.03 ±0.02[a]	2.05 ±0.03[a]
Fat, %	0.98 ±0.02[a]	1.02 ±0.05[a]	0.99 ±0.01[a]	0.98 ±0.04[a]
Total sulfhydryl groups, µmol/g	27.13 ±1.05[c]	35.45 ±0.94[a]	31.04 ±1.45[b]	23.67 ±1.56 [d]
Free sulfhydryl groups, µmol/g	5.44 ±0.07[a]	4.92 ±0.11[c]	4.98 ±0.08[c]	5.13 ±0.06[b]
Disulfide bond,SS contents, µmol/g	10.84 ±0.19[c]	15.26 ±0.07[a]	13.03 ±0.23[b]	9.27 ±0.60[d]

a–d – row values followed by the same superscript letter are not significantly different ($p < 0.05$).
*Means ±standard deviation values of triplicates.

index (CI), as well as Hausner ratio (HR), ranged from 11.94 to 15.39 and 1.10 to 1.18, respectively. These values indicate that the GSPC powder exhibited satisfactory flowability. The CI values of GSPC were in the range of 11 to 15, which is considered as 'good' in terms of flowability. The flow behaviour is significantly influenced by the surface composition, as flowability requires overcoming the interparticle surface attractions. The HR values observed in this investigation were similar to the HR values reported for milk powder in a study reported by Fitzpatrick et al. (2007). The angle of repose is employed to determine the degree of dispersion of dry powdered materials. Good dispersibility is indicated by a low angle of repose. GSPC powder exhibited the shortest angle of repose at a pH of 9, which can be attributed to the particles' spherical shape, low friction, and high fluidity.

Wettability and water activity (a_w)

Wettability is a measure that quantifies the time it takes for protein samples to absorb water and become fully saturated. The wettability value of the GSPC samples ranged from 14.03 to 9.11 min. The poor wettability value could be attributed to the presence of highly hydrophilic regions in the protein structure. GSPC powder has an a_w value ranging from 0.48 to 0.51 (Table 1). The protein sample is meant to function as a low-moisture product that can be stored for a long time without refrigeration. The results obtained for all the protein samples were consistent with the findings of Zhang et al. (2019). At a pH of 8, the GSPC sample showed the lowest water activity, suggesting a stability in its quality and its suitability for storage.

Functional properties

The functional properties of food items have a significant impact on their processing, storage, and organoleptic properties. The protein solubility (PS) increased from 82.07% to 92.01% when the extraction pH was raised to 11 (Fig. 1A). This could be attributed to the decreased particle size and reduced surface hydrophobicity of GSPC samples. The process of dissociation,

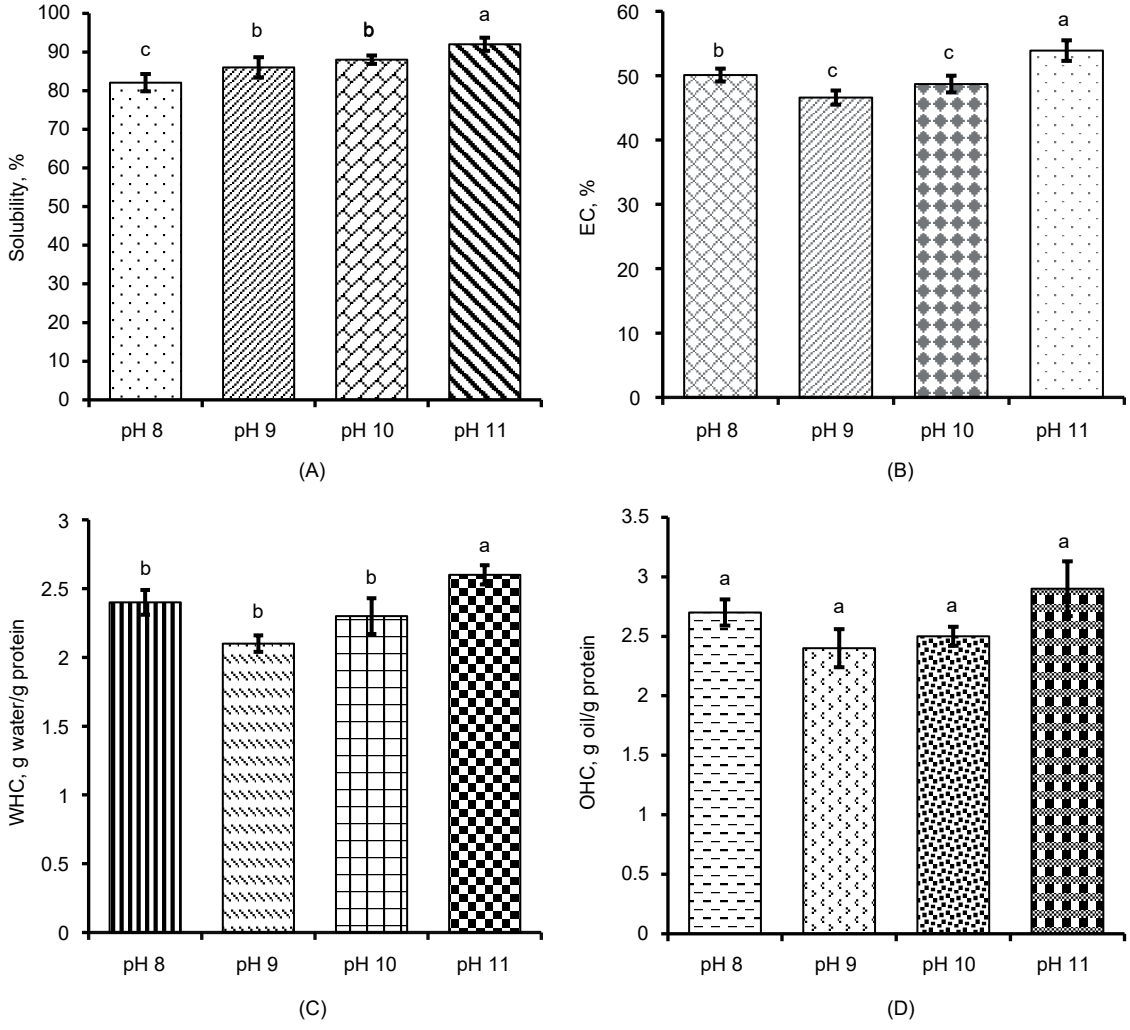

Fig. 1. Effect of different extraction pH on (A) solubility (%), (B) emulsion capacity (%), (C) water holding capacity (g/g), and (D) oil holding capacity (g/g) of GSPC

in which amino acids separate into H+ and $–NH_2$ ions, COOH disintegrates into H^+ and $–COO^–$ ions, and neutral and acidic amino acids ionise, likely had a significant impact on the protein solubility (Kamani et al., 2023).

The emulsifying capacity (EC) is a measure of how protein molecules interact with water and lipid molecules, particularly in areas with a high concentration of polar side chains (Kamani et al., 2023). Protein molecules contribute to the stabilisation of the water-lipid interface by decreasing interfacial tension and creating a thin layer around the lipid molecules. This improves the viscosity of the continuous phase. EC of GSPC samples exhibited a reduction from 50.10% to 46.60% when the extraction pH increased from 8 to 9. Subsequently, the EC increased to 53.90% at pH 11 (Fig. 1B). The initial decrease in EC could be linked to an increase in β-sheet formations and a decrease in surface hydrophobicity. Due to the enhanced Coulombic connections, the protein molecules might have potentially established a robust network, leading to an increase in the EC (Mir et al., 2019). Meanwhile, an increase in protein solubility, a decrease in particle size, and the presence of hydrophilic interactions all showed a favourable correlation with EC (Deb et al., 2022). Vuong et al. (2016) found that the concentrate from rambutan seed with an increased surface charge, as well as PS, exhibited higher EC as a result of improved stability at the water-oil interface.

The water holding capacity (WHC) of GSPC samples was significantly affected by the extraction pH (Fig. 1C). Furthermore, the differences in water holding capacity (WHC) may be linked to the adaptable structure, presence of polar and charged regions in the protein chain, and hydrophobic characteristics (Kumar et al., 2022). The oil holding capacity (OHC) is crucial for preserving the taste as well as texture characteristics of a product (Kamani et al., 2023). Amino groups that lack polarity tend to form bonds with lipid chains (aliphatic), which are representative of the OHC (Shevkani et al., 2019). The OHC of GSPC samples ranged from 2.4 to 2.9 (g oil/g protein), as shown in Fig. 1D. The increase in OHC at elevated extraction pH indicates the presence of non-polar side chains (Kumar et al., 2022). Foams are mainly produced through the diffusion of globular proteins into the interface between water and air, via changes in concentration, and through the reduction of surface tension (Kamani et al., 2023). Subsequently, the peptides undergo unfolding, resulting in the formation of a protective layer at the contact point (Kumar et al., 2022). The foaming capacity (FC) of GSPC samples exhibited substantial variation ($p < 0.05$), ranging from 2.2 to 8.0 mL (Table 1). The lowest FC of 2.2 mL was observed at pH 8. Protein molecules that have a smaller particle size, greater surface charge, more soluble fractions, and highly flexible structures have stronger repulsive forces along with weaker hydrophobic interactions. This prevents bubbles from merging and results in better foam stability (Deb et al., 2022). Alvarez-Ossorio et al. (2022) obtained a grape protein concentrate that exhibited a WHC ranging from 1.91 to 4.47 (g water/g protein), and an OHC ranging from 1.94 to 4.67 (g oil/g protein).

Sulfhydryl group (SH) and disulphide bonds (SS)

Disulphide bonds (SS) are strong connections formed between cysteine residues. These bonds play a crucial role in preserving the intricate three-dimensional structure of proteins. With an increase in the extraction pH from 8 to 9, the GSPC samples exhibited a rise in both SS and Total SH content, from 10.84 to 15.26 μmol/g and from 27.13 to 35.45 μmol/g, respectively (Table 1). The possible cause could be the side chain deprotonation of cysteine residues, leading to the generation of reactive thiol groups and subsequent formation of SS bonds. Moreover, an increased presence of disulphide linkages within protein molecules is indicative of a larger concentration of SS. Nevertheless, the SS and SH content decreased to 9.27 and 23.67 μmol/g, respectively, when the pH increased from 10 to 11. Such occurrences may be accountable for the SH group's protonation, leading to a limitation in the formation of SS bonds. Nevertheless, the rise in cysteine concentration in GSPC samples also led to an increase in the number of SS groups at pH 9. At a pH greater than 9, it is possible that cysteine residues have developed thiolate groups. However, these thiolate groups are vulnerable to degradation, which can result in a decrease in the SS bond. However, the levels of free sulfhydryl groups in GSPC samples decreased from 5.44 to 4.92 (μmol/g) when the extraction pH was raised from 8 to 9, and then subsequently increased to 5.13 (μmol/g). Primarily, enhanced intermolecular connections led to the reduction of the free sulfhydryl groups,

consequently yielding a denser structure. Concurrently, the increased availability of unbound sulfhydryl groups prompted the development of breaks in the polypeptide chain, potentially expediting the process of protein unfolding (Liu et al., 2021). Gao et al. (2020) supported the current results in their study on pea protein. They found that changing the extraction pH from 8.5 to 9.5 resulted in a decrease of free sulfhydryl groups by 16.78%, while simultaneously enhancing the SH groups by 15.86% and SS content by 24.76%.

Colour

The GSPC obtained displayed a brownish colour, as evidenced by the *L*a*b** colour pattern (Table 2). The GSPC had a low lightness value (*L** value), while both the positive *a** and *b** values confirmed the brown look. The GSPC colour profile was significantly influenced ($p < 0.05$) by a pH modification. The chroma values increased proportionally with a rise in the pH, indicating that the colour of the GSPC became more saturated. *ΔE** represents the overall colour difference, ranging from nothing changed (0) to an entire change (100). *ΔE** value showed a maximum value at pH 10. A dark-coloured protein concentrate was obtained by extracting protein at alkaline pH levels ranging from 8 to 11. The covalent interaction of phenolic compounds with proteins in the grape seed protein matrix is considered to promote the oxidation of these compounds, leading to the browning of the concentrate. This phenomenon is especially noticeable in alkaline conditions (Diosady et al., 1987). A comparable outcome, specifically a lower *L** value, was reported in studies conducted by Garg et al. (2020) on sangri seed protein concentrate.

Amino acid and nutritional parameters

GSPC samples have high levels of glutamic acid, aspartic acid, arginine, serine, and phenylalanine (Table 3). When the extraction pH was increased from 8 to 11, the cysteine content reached its peak at pH 9, with a value of 8.2 mg/g, while the histidine content reached its highest level at pH 10, with a value of 45.1 mg/g. In addition, the total amount of essential amino acids (EAA) exceeded the recommended standards set by FAO/WHO (2013), which is 275 mg/g of protein. The EAA content also showed significant variation depending on the extraction pH, with the highest values recorded at pH 9. The non-essential amino acids, in this case, glycine, aspartic acid, glutamic acid, and proline were found to have the highest concentrations. The levels of serine, glutamic acid, and alanine in GSPC samples reached their highest values at pH 10. The GSPC samples had significantly ($p < 0.05$) different total amino acid contents of 911.9, 964.6, 982.6, and 917.1 (mg/g) at pH levels 8, 9, 10, and 11, respectively. However, total amino acids content of 911.9 (mg/g) observed at pH 8 was statistically the most divergent. For every scenario, the E/T ratios (ratio of total essential amino acids to total amino acid levels) exceeded 36%, which suggests an optimal protein source (Ge et al., 2021). Furthermore, it is worth noting that all the GSPC samples exhibited a greater concentration of aromatic amino acids compared to the limits set by FAO/ WHO in 2013, which stipulated a minimum level of 41 mg/g. Similarly, the overall levels of amino acids containing sulphur in all the GSPC samples met the criteria set by FAO/WHO (2013), with the exception of those recorded at pH 8. Conversely, raising the extraction pH from

Table 2. Color characteristics of grape seed protein concentrate extracted at different pH

Treatments	Parameters					
	*L**	*a**	*b**	*ΔE**	*Hue°*	*Chroma*
pH 8	9.0 ±0.23^{d}	14.1 ±0.12^{c}	37.7 ±0.09^{d}	41.24 ±0.32^{d}	22.49 ±0.15^{a}	40.24 ±0.53^{d}
pH 9	10.9 ±0.17^{c}	13.8 ±0.26^{d}	38.7 ±0.14^{c}	42.50 ±0.09^{c}	19.28 ±0.17^{b}	41.09 ±0.34^{c}
pH 10	16.6 ±0.24^{b}	20.9 ±0.45^{a}	47.6 ±0.13^{a}	54.57 ±0.98^{a}	19.42 ±0.23^{b}	51.94 ±0.34^{a}
pH 11	17.1 ±0.13^{a}	17.3 ±0.11^{b}	46.0 ±0.18^{b}	52.03 ±0.21^{b}	15.64 ±0.09^{c}	49.12 ±0.13^{b}

a–d – column values followed by the same superscript letter are not significantly different ($p < 0.05$).
*Means ±standard deviation values of triplicates.

Table 3. Amino acid profile of GSPC obtained at different pH of extraction

Essential Amino Acids mg/g	pH 8	pH 9	pH 10	pH 11	Adult requirement (FAO/WHO, 2013)
Threonine	58.9 ±0.15^{c}	54.2 ±0.08^{d}	66.1 ±0.23^{a}	64.4 ±0.01^{a}	25
Cysteine	7.5 ±0.02^{a}	8.2 ±0.03^{a}	6.6 ±0.0^{c}	5.9 ±0.02^{d}	
Valine	16.8 ±0.02^{a}	15.8 ±0.02^{c}	11.2 ±0.09^{d}	12.2 ±0.02^{c}	40
Methionine	11.0 ±0.03^{b}	18.4 ±0.01^{a}	18.1 ±0.04^{a}	17.7 ±0.06^{a}	
Isoleucine	39.1 ±0.05^{b}	40.1 ±0.07^{b}	45.6 ±0.06^{a}	39.7 ±0.09^{b}	30
Leucine	27.3 ±0.17^{b}	28.9 ±0.01^{a}	29.9 ±0.07^{a}	26.5 ±0.04^{c}	61
Tryptophan	30.0 ±0.09^{d}	35.0 ±0.05^{c}	37.0 ±0.12^{b}	58.0 ±0.02^{a}	6.6
Phenylalanine	40.4 ±0.07^{c}	46.4 ±0.03^{b}	47.5 ±0.02^{a}	31.9 ±0.05^{d}	
Lysine	52.9 ±0.07^{a}	54.0 ±0.09^{c}	35.9 ±0.01^{d}	36.3 ±0.01^{c}	48
Histidine	31.1 ±0.08^{d}	41.3 ±0.04^{b}	45.1 ±0.06^{a}	35.8 ±0.02^{c}	16
Tyrosine	30.1 ±0.02^{c}	34.5 ±0.05^{b}	39.0 ±0.09^{a}	24.6 ±0.02^{d}	
EAA	345.2 ±0.76^{c}	376.8 ±0.48^{b}	382.0 ±0.82^{a}	353.0 ±0.35^{c}	275
Non-Essential Amino Acids					
Aspartic Acid	72.0 ±0.02^{c}	73.3 ±0.04^{b}	77.1 ±0.02^{a}	73.2 ±0.03^{b}	
Serine	42.3 ±0.01^{c}	44.3 ±0.03^{b}	47.8 ±0.08^{a}	40.7 ±0.03^{d}	
Glutamic Acid	209.0 ±0.16^{c}	211.7 ±0.13^{b}	216.0 ±0.09^{a}	211.1 ±0.07^{b}	
Glycine	81.1 ±0.03^{d}	87.3 ±0.04^{b}	89.1 ±0.03^{a}	85.5 ±0.02^{c}	
Alanine	46.0 ±0.02^{c}	48.7 ±0.01^{b}	49.5 ±0.02^{a}	40.3 ±0.02^{d}	
Arginine	77.1 ±0.04^{a}	75.0 ±0.03^{b}	72.7 ±0.04^{c}	72.5 ±0.03^{c}	
Proline	38.3 ±0.01^{b}	39.7 ±0.02^{a}	37.3 ±0.02^{c}	35.0 ±0.03^{d}	
NEAA	565.8 ±0.29^{c}	580.0 ±0.30^{b}	589.5 ±0.29^{a}	558.3 ±0.23^{d}	
Total AA	911.9 ±1.05^{d}	964.6 ±0.78^{b}	982.6 ±1.11^{a}	917.1 ±0.58^{c}	
E/T, %	37.8	39.0	38.8	38.4	
PER 1	0.38 ±0.06^{b}	0.44 ±0.04^{ab}	0.50 ±0.02^{a}	0.35 ±0.04^{b}	
PER 2	0.45 ±0.02^{a}	0.48 ±0.01^{a}	0.47 ±0.02^{a}	0.47 ±0.03^{a}	
Total sulphur containing amino acids	18.5	26.6	24.7	23.6	
Total neutral amino acids	274.1	289.0	299.2	267.7	
Total aromatic amino acids	100.5	115.9	123.5	114.5	
Total acidic amino acids	281.0	285.0	293.1	284.3	
Total basic amino acids	161.1	170.3	153.7	144.6	
Leucine/isoleucine ratio	6.9	6.3	6.5	6.6	
Lysine/arginine ratio	6.8	7.2	4.9	5.0	
Total hydroxylic amino acids	101.2	98.5	113.9	105.1	

a–d – column values followed by the same superscript letter are not significantly different ($p < 0.05$).
*Means ±standard deviation values of triplicates.

8 to 10 enhanced the overall concentration of neutral amino acids, after which it decreased at higher pH levels. In addition, the increased presence of hydrophobic amino acids led to the development of a compact central region with a spherical shape, enhancing both the stability and surface functionality of the protein (Liu et al., 2021). When the extraction pH is increased, the ionizable groups present in protein molecules acquire a greater positive charge. This leads to the denaturation of the protein and an increase in solubility. Consequently, new amino acid residues are exposed. This phenomenon has been previously supported by Kheto et al. (2024). An increase in extraction pH could potentially trigger oxidation and hydrolysis reactions, leading to the degradation of amino acids (AA). The nutritional composition of GSPC samples was assessed using the protein efficiency ratio (PER). PER is a measure of protein quality and is calculated by dividing the weight gained by the amount of protein consumed over a specific time period. PER values of GSPC samples at pH levels 8, 9, 10, and 11 ranged from 0.35 to 0.50, suggesting a high protein quality (Ge et al., 2021).

Differential scanning calorimetry (DSC)

The thermal stability of GSPC samples was assessed by employing DSC to investigate their denaturation and aggregation characteristics. The denaturation temperatures of the GSPC samples at pH 8, 9, 10, and 11 were measured to be 86.28, 98.26, 103.32, and 104.23°C, respectively (Fig. 2A). These results indicate that the protein molecules had a tightly packed tertiary structure. The temperature of denaturation is linked to the presence of β-sheet structure as well as the amount of hydrophobic amino acids (Liu et al., 2021). The analysis revealed that the denaturation temperature reached its peak at pH 11. Additionally, this particular sample exhibited a high concentration of hydrophobic amino acids and a significant presence of β-sheet structure. Raising the pH during extraction resulted in a progressive increase in denaturation temperature, from 86.28°C to 104.23°C. The increased denaturation temperature may be attributed to the enhanced intermolecular interactions facilitated by the exposed hydrophilic as well as hydrophobic regions in the polypeptide chain. The enthalpy of denaturation (ΔH) corresponds to the breakdown of a given organised structure during an endothermic reaction. It was discovered that the change in enthalpy (ΔH) was influenced by the level of purity and the pH of the extraction process. Lowering the pH during the extraction process resulted in a drop in ΔH to 4.61 (J/g), which could be attributed to the occurrence of partial unfolding. Similarly, according to Song et al. (2023), raising the extraction pH level from 8 to 10 resulted in an improvement in the temperature of denaturation in soybean oil-body wastewater protein, increasing it from

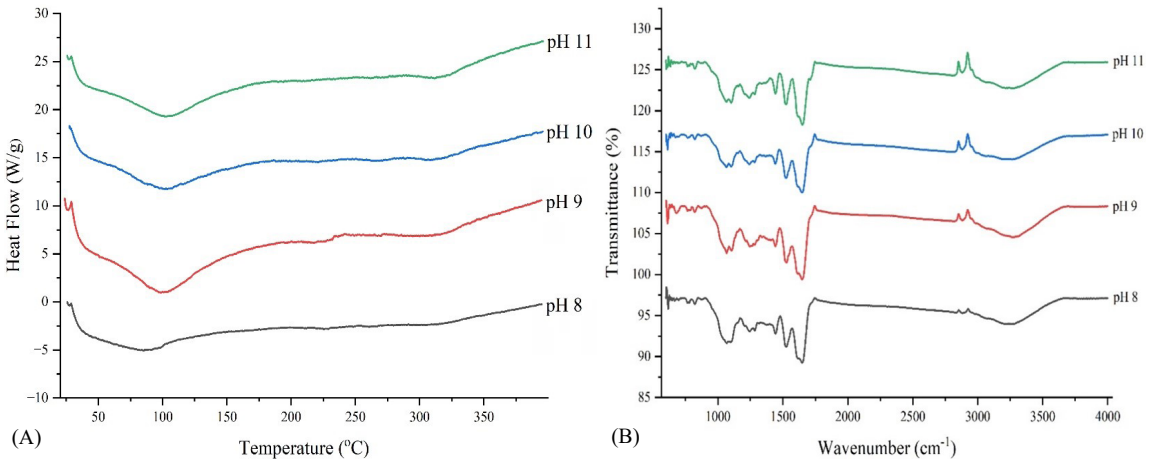

Fig. 2. Effect of different extraction pH on (A) Differential scanning calorimetry (W/g), (B) FTIR Spectra (%) of GSPC

89.96 to 92.95°C. However, the denaturation temperature fell at pH 11, reaching 86.95°C. On the flip side, Liu et al. (2021) found that raising the extraction pH (from 8 to 9), in the case of mung bean protein, resulted in a decrease in the denaturation temperature, from 77.61°C to 75.1°C, as a consequence of protein unfolding.

FTIR

The FTIR spectra of GSPC samples displayed minor fluctuations in peak strength and absorbance patterns (Fig. 2B). The spectral range of 3100–3500 cm^{-1} corresponds to the bending of O–H bonds and stretching of N–H in amide A. This range also includes the influence of water molecules on these bonds. The peak intensity, within this range, is highest at 3280 cm^{-1}, as reported by Figueroa-Gonzalez et al. (2022). Peak intensities were found to vary as the extraction pH increased from 8 to 11. This phenomenon can be ascribed to the enhancement or deterioration of hydrogen bonding inside protein molecules. The spectral range of 2900–3000 cm^{-1} was attributed to amide B, which involves the symmetric as well as asymmetric vibrations of $–CH_3$ and $–CH_2$ groups. This observation was also made by Figueroa-Gonzalez et al. (2022), with a prominent peak observed at 2930 cm^{-1}. In addition, the spectral areas between 1600–1700 cm^{-1} correspond to the C–O bonds stretching (80%) and N–H bonds bending (20%) in the amide I region (Shevkani et al., 2019). This region had the highest peak strength at 1638 cm^{-1}, indicating that the extraction pH had a considerable impact on the bending and stretching of N–H and C–H bonds, respectively, as compared to other regions. Furthermore, elevating the pH during extraction caused the amino acids to undergo deprotonation, resulting in a rise in molecular polarity and a weakening of hydrogen bonding. Consequently, this led to a decrease in peak intensity. In addition, the larger peak area observed in GSPC samples suggests that there are structural disparities in the protein molecules, which can be attributed to differences in hydrogen bonding, electrostatic interactions, and repulsive forces. Furthermore, a distinct peak at 1523 cm^{-1} was seen within the range of 1500–1600 cm^{-1}, which suggests that the N-H as well as C-N bonds were bending in the amide II area. Similarly, a little depression was observed at 1450 cm^{-1}, indicating the C–H bonds bending. The regions between 1200–1300 cm^{-1}, specifically around 1228 cm^{-1}, were associated with the stretching (40%) and bending (30%) of C–N as well as N–H bonds in amide III regions. These regions also exhibited interactions with various other macromolecules, as reported. Of all the amide groups, amide III exhibited a high degree of peak intensity.

Morphological characteristics

The microstructure of the GSPC-tailored sample (Fig. 3) under high alkaline conditions exhibited reduced disintegration and a smoother appearance. This phenomenon can be attributed to the heightened presence of interaction forces under alkaline conditions, leading to the formation of compact aggregates and resulting in a smooth structural appearance. The microstructure of sample (D) exhibited a higher degree of smoothness compared to other pH-tailored GSPCs. This could be attributed to the fact that the sample (D) preparation was conducted under more alkaline conditions (pH 11) compared to the other samples. The protein underwent unfolding due to the high alkaline pH, leading to an increase in the exposure of hydrophobic as well as sulfhydryl groups. This, in turn, caused the protein to aggregate and exhibit a smooth structural appearance. Gao et al. (2019) also made similar conclusions in their work on whey protein, which was prepared under different pH conditions.

CONCLUSIONS

The intermolecular interactions, nutritional characteristics, thermal properties, and functional characteristics of GSPC were analysed at different extraction pH levels. The elevated extraction pH resulted in an enhanced yield but also decreased protein purity. GSPC extracted at pH 11 exhibited most of the superior functional characteristics compared to the other samples. GSPCs demonstrated substantial amounts of total essential amino acids (EAA), hydroxylic and aromatic amino acids. GSPCs showed a higher total essential amino acid content compared to hemp, wheat, lupin and oats. All of the GSPCs had comparable solubility and emulsifying potential, while exhibiting lower foaming properties compared to soy protein. The colour of GSPCs was noticeably more brown and deeper. These GSPCs have the potential to serve as colour

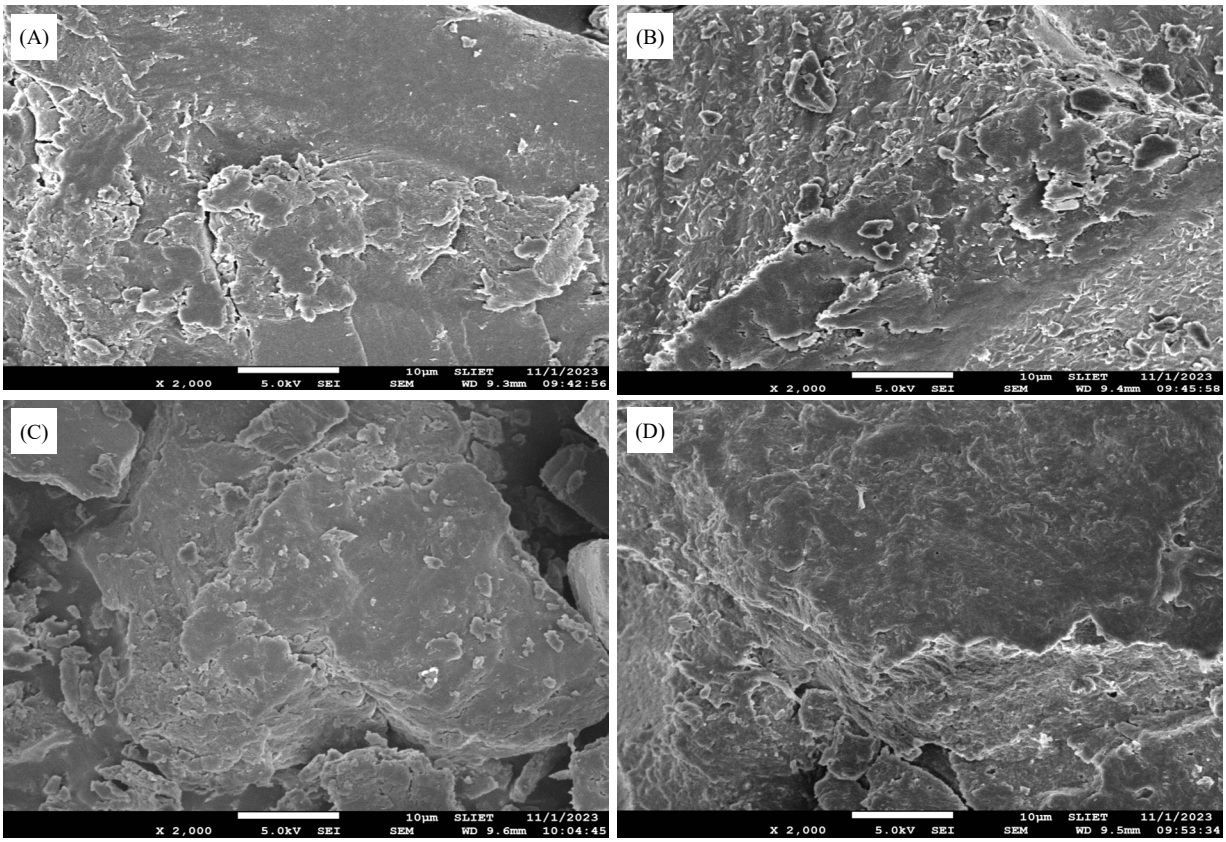

Fig. 3. Effect of different extraction pH on morphological characteristics (×2000) of GSPC evaluated by scanning electron microscopy, A) pH 8; B) pH 9; C) pH 10; and D) pH 11

stabilisers as well as agents for improving the clarity of wine in industrial processes. This might be a significant advancement in the field of industrial winemaking, particularly for wines produced in warm climates that are prone to losing colour. The denaturation temperature of GSPCs was greater than that of sunflower globulins. Foods containing proteins that undergo denaturation at approximately 100°C can be exposed to baking, boiling, as well as other cooking techniques at high temperatures, without experiencing any loss in their structural integrity. This attribute is essential for obtaining an acceptable texture and uniformity in several culinary uses. Optimal flow properties are essential for achieving homogeneous blending and mixing of GSPCs with other components, hence ensuring consistent product quality. The flow characteristics of protein concentrates are crucial for their efficient utilisation in many food formulations, along with other industrial applications. The functional characteristics of GSPC suggest that it has the potential to enhance desirable characteristics in various food products. GSPC is suitable for incorporation in dairy and pastry products. Hence, GSPC exhibits the capacity to be employed in the food processing sector as a functional food additive. Further investigation is needed to improve the functional properties of extracted GSPC by applying appropriate non-thermal technologies.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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REFERENCES

Alvarez-Ossorio, C., Orive, M., Sanmartín, E., Alvarez-Sabatel, S., Labidi, J., Zufia, J., Bald, C. (2022). Composition and techno-functional properties of grape seed flour protein extracts. ACS Food Sci. Technol., 2(1), 125–135. https://doi.org/10.1021/acsfoodscitech.1c00367

Arogundade, L. A., Tshay, M., Shumey, D., Manazie, S. (2006). Effect of ionic strength and/or pH on extractability and physico-functional characterization of broad bean (*Vicia faba* L.) protein concentrate. Food Hydrocoll., 20(8), 1124–1134. https://doi.org/10.1016/j.foodhyd.2005.12.010

Baca-Bocanegra, B., Martínez-Lapuente, L., Nogales--Bueno, J., Hernández-Hierro, J. M., Ferrer-Gallego, R. (2021). Feasibility study on the use of ATR-FTIR spectroscopy as a tool for the estimation of wine polysaccharides. Carbohydr. Polym., 287, 119365. https://doi.org/10.3390/foods10010079

Chinta, D. D., Graves, R. A., Pamujula, S., Praetorius, N., Bostanian, L. A., Mandal, T. K. (2009). Spray-dried chitosan as a direct compression tableting excipient. Drug Dev. Ind. Pharm., 35(1), 43–48. https://doi.org/10.1080/03639040802149053

Deb, S., Kumar, Y., Saxena, D. C. (2022). Functional, thermal and structural properties of fractionated protein from waste banana peel. Food Chem.: X., 100205. https://doi.org/10.1016/j.fochx.2022.100205

Diosady, L. L., Tar, C. G., Rubin, L. J., Naczk, M. (1987). Scale-up of the production of glucosinolate-free canolameal. Acta Aliment., 16(2), 167–179.

FAO/WHO (2013). Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. Food and Nutrition Paper No. 92. Rome: Food and Agriculture Organization and the World Health Organization.

FAOSTAT (2021). FAOSTAT Crops Statistics http://www.fao.org/faostat/en/#data/QC (accessed January 20, 2021).

Figueroa-González, J. J., Lobato-Calleros, C., Vernon-Carter, E. J., Aguirre-Mandujano, E., Alvarez-Ramirez, J., Martínez-Velasco, A. (2022). Modifying the structure, physicochemical properties, and foaming ability of amaranth protein by dual pH-shifting and ultrasound treatments. LWT – Food Sci. Technol., 153, 112561. https://doi.org/10.1016/j.lwt.2021.112561

Fitzpatrick, J. J., Barry, K., Cerqueira, P. S. M., Iqbal, T., O'Neill, J., Roos, Y. H. (2007). Effect of composition and storage conditions on the flowability of dairy powders. Int. Dairy J., 17(4), 383–392. https://doi.org/10.1016/j.idairyj.2006.04.010

Freudig, B., Hogekamp, S., Schubert, H. (1999). Dispersion of powders in liquids in a stirred vessel. Chem. Eng. Process.: Process Intensif., 38(4-6), 525–532. https://doi.org/10.1016/S0255-2701(99)00049-5

Gao, H., Ma, L., Li, T., Sun, D., Hou, J., Li, A., Jiang, Z. (2019). Impact of ultrasonic power on the structure and emulsifying properties of whey protein isolate under various pH conditions. Process Biochem., 81, 113–122. https://doi.org/10.1016/j.procbio.2019.03.012

Gao, Z., Shen, P., Lan, Y., Cui, L., Ohm, J. B., Chen, B., Rao, J. (2020). Effect of alkaline extraction pH on structure properties, solubility, and beany flavor of yellow pea protein isolate. Food Res. Int., 131, 109045. https://doi.org/10.1016/j.foodres.2020.109045

Garg, D., Chakraborty, S., Gokhale, J. S. (2020). Optimizing the extraction of protein from *Prosopis cineraria* seeds using response surface methodology and characterization of seed protein concentrate. LWT – Food Sci. Technol., 117, 108630. https://doi.org/10.1016/j.lwt.2019.108630

Ge, J., Sun, C. X., Mata, A., Corke, H., Gan, R. Y., Fang, Y. (2021). Physicochemical and pH-dependent functional properties of proteins isolated from eight traditional Chinese beans. Food Hydrocoll., 112, 106288. https://doi.org/10.1016/j.foodhyd.2020.106288

Kamani, M. H., Neji, C., Fitzsimons, S. M., Fenelon, M. A., Murphy, E. G. (2023). Unlocking the nutritional and functional potential of legume waste to produce protein ingredients. Crit. Rev. Food Sci. Nutr., 3, 1–19. https://doi.org/10.1080/10408398.2023.2184322

Kheto, A., Sehrawat, R., Gul, K., Kumar, L. (2024). Effect of extraction pH on amino acids, nutritional, *in-vitro* protein digestibility, intermolecular interactions, and functional properties of guar germ proteins. Food Chem., 444, 138628. https://doi.org/10.1016/j.foodchem.2024.138628

Kornet, R., Roozalipour, S. L., Venema, P., van der Goot, A. J., Meinders, M. B., van der Linden, E. (2022). Coacervation in pea protein solutions: The effect of pH, salt, and fractionation processing steps. Food Hydrocoll., 125, 107379. https://doi.org/10.1016/j.foodhyd.2021.107379

Kumar, M., Tomar, M., Potkule, J., Punia, S., Dhakane-Lad, J., Singh, S., Kennedy, J. F. (2022). Functional characterization of plant-based protein to determine its quality for food applications. Food Hydrocoll., 123, 106986. https://doi.org/10.1016/j.foodhyd.2021.106986

Lawal, O. S., Adebowale, K. O., Adebowale, Y. A. (2007). Functional properties of native and chemically modified protein concentrates from bambarra groundnut. Food Res. Int., 40, 1003–1011. https://doi.org/10.1016/j.foodres.2007.05.011

Liu, F. F., Li, Y. Q., Wang, C. Y., Zhao, X. Z., Liang, Y., He, J. X., Mo, H. Z. (2021). Impact of pH on the physicochemical and rheological properties of mung bean (*Vigna radiata* L.) protein. Process Biochem., 111, 274–284. https://doi.org/10.1016/j.procbio.2021.10.008

Mir, N. A., Riar, C. S., Singh, S. (2019). Structural modification of quinoa seed protein isolates (QPIs) by variable time sonification for improving its physicochemical and functional characteristics. Ultrason. Sonochem., 58, 104700. https://doi.org/10.1016/j.ultsonch.2019.104700

Salgado, P. R., Molina Ortiz, S. E., Petruccelli, S., Mauri, A. N. (2011). Sunflower protein concentrates and isolates prepared from oil cakes have high water solubility and antioxidant capacity. J. Am. Oil Chem. Soc., 88, 351–360. https://doi.org/10.1007/s11746-010-1673-z

Sheikh, M. A., Saini, C. S., Sharma, H. K. (2022). Synergistic effect of microwave heating and hydrothermal treatment on cyanogenic glycosides and bioactive compounds of plum (*Prunus domestica* L.) kernels: An analytical approach. Curr. Res. Food Sci., 5, 65–72. https://doi.org/10.1016/j.crfs.2021.12.007

Shevkani, K., Singh, N., Chen, Y., Kaur, A., Yu, L. (2019). Pulse proteins: Secondary structure, functionality and applications. J. Food Sci. Technol., 56, 2787–2798. https://doi.org/10.1007/s13197-019-03723-8

Sofi, H. S., Akram, T., Shabir, N., Vasita, R., Jadhav, A. H., Sheikh, F. A. (2021). Regenerated cellulose nanofibers from cellulose acetate: Incorporating hydroxyapatite (HAp) and silver (Ag) nanoparticles (NPs), as a scaffold for tissue engineering applications. Mater. Sci. Eng. C., 118, 111547. https://doi.org/10.1016/j.msec.2020.111547

Song, H., Zhong, M., Sun, Y., Li, Y., Qi, B. (2023). Recovery of proteins from soybean oil-body wastewater at various pH levels and their structural and functional characterization. LWT – Food Sci. Technol., 174, 114455. https://doi.org/10.1016/j.lwt.2023.114455

Vuong, H. T. H., Tran, N. M. C., Tran, T. T. T., Ton, N. M. N., Le, V. V. M. (2016). Effects of pH and salt concentration on functional properties of rambutan (*Nephelium lappaceum* L.) seed albumin concentrate. Int. J. Food Sci., 51, 1212–1219. https://doi.org/10.1111/ijfs.13087

Zhang, Z., Zhang, R., Xiao, H., Bhattacharya, K., Bitounis, D., Demokritou, P., McClements, D. J. (2019). Development of a standardized food model for studying the impact of food matrix effects on the gastrointestinal fate and toxicity of ingested nanomaterials. NanoImpact., 13, 13–25. https://doi.org/10.1016/j.impact.2018.11.002

Zumwalt, R. W., Absheer, J. S., Kaiser, F. E., Gehrke, C. W. (1987). Acid hydrolysis of proteins for chromatographic analysis of amino acids. J. Assoc. Off. Anal. Chem., 70, 147–151. https://doi.org/10.1093/jaoac/70.1.147