

Valorization of noni (*Morinda citrifolia*) seeds as source of a protein concentrate and its physicochemical, funcional and structural characterization

Jorge Adrián Hernández Ramírez

Universidad Autónoma de Nayarit: Universidad Autonoma de Nayarit

José Armando Ulloa (✉ arulloa5@gmail.com)

Universidad Autónoma de Nayarit: Universidad Autonoma de Nayarit <https://orcid.org/0000-0002-3749-3086>

Blanca Estela Ulloa Rangel

Universidad Autónoma de Nayarit: Universidad Autonoma de Nayarit

Petra Rosas Ulloa

Universidad Autónoma de Nayarit: Universidad Autonoma de Nayarit

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Abstract

The noni juice industry produces a large volume of seeds as by-products, which contain proteins that can be recovered to be used as food ingredients. The aims of this study were the valorization of noni seeds as source of a protein concentrate (NSPC) and their physicochemical, functional, and structural characterization. NSPC was produced by alkaline extraction and isoelectric precipitation, obtaining a powder with a protein content of 76.59%, whose values of the color parameters were $L^* = 55.32\%$, $a^* = 4.60\%$, and $b^* = 19.09$. Water ($WHCa$) and oil ($OHCa$) holding capacities of the NSPC were 4.36 g H_2O /g protein and 11.69 g oil/g protein, respectively, while the emulsifying activity, emulsion stability, foaming capacity, foaming stability, least gelling concentration, and *in vitro* digestibility were, 29.20%, 50.00%, 180.33%, 94.60%, 4.00%, and 78.51%. Glutelins (64.62%) were the main protein fraction of the NSPC. Hence, NSPC could be used as ingredient for human food products as bread, soups, salad dressings, mayonnaise, and processed meat products.

Introduction

The most recent report of The United Nations' Food and Agricultural Organization state that the fruit production in the world for the 2020 year was 887 millions of ton, which represented 9.5% of the production of primary crops [1]. Unfortunately, about 30% of fruit and vegetable production is lost after harvesting due to spoilage with the consequent environmental impact [2]. On the other hand, fruit processing industries produce by-products such as peels and seeds from their raw materials, which constitutes an environmental risk since they are generally disposed of as waste by the sanitary landfill method [3]. The disposal of waste through sanitary landfills severely affects the ecology due to the emission of methane into the atmosphere, as a consequence of anaerobic digestion and the production of leachates, which drain to the bottom of the earth during the rainstorm [4]. In particular, the global fruit processing industry generates more than 0.5 billion tons of waste [5].

Fruit processing wastes, which cause contamination problems when not properly treated, also contain various nutrients and bioactive compounds that can be recovered as high-value novel products for use as food ingredients [6–7]. A wide variety of molecules including proteins, lipids, sugars, fiber, minerals, vitamins, pigments, and antioxidants can be extracted from the by-products of fruit processing [8]. According to some recent studies, fruit seeds as by-products from processing of jackfruit [9], passion fruit [10], mango [11], guamuchil [12], and orange [13] have been utilized to obtain protein concentrates or isolates of great functional quality, which could be used in the food industry as food ingredients, as well as functional foods or design of new products.

To date there is a clear trend among consumers towards foods of vegetable origin, especially those of a protein nature, and their contribution in the market is increasingly important because they are cheaper, healthier, and satisfy religious, moral, or environmental aspects that are not achieved with the consumption of ingredients of animal origin [14]. In this sense, vegetable proteins have been used successfully for the preparation of meat, egg, milk, and cheese analogues [15]. The successful

application of protein ingredients in the development of food products depends on their functional properties, such as solubility, water and oil absorption capacities, and gelling, foaming and emulsifying properties, etc. [16], which must be evaluated to explore the potential application of a proteins source new.

Morinda citrifolia L. or noni is a perennial fruit plant up to 6 m tall and appertains to the Rubiaceae family, which is native of Polynesia and is currently cultivated in tropical and subtropical regions of America, China, Vietnam, India, Malaysia, and Cambodia [17]. Noni fruits have been widely used in traditional medicine to treat various diseases such as hypertension, tuberculosis, diabetes, and arthritis, but more recent studies have shown its usefulness in cancer treatments [18]. The most important industrialized product of the noni fruit is the juice, which has gained worldwide popularity because the consuming population has perceived beneficial effects on health, such as the reduction of pain and allergy and asthma symptoms, improved sleep, digestion, and well-being, as well as more energy and fewer infections [19]. Noni juice together with other products from such fruit represent a market value of US \$ 1.5 billion [20] of estimated total of \$ US \$651.2 billion of world market of functional foods by 2021 [21].

The main residue from the processing of the noni fruit is the seeds, which represent approximately 2.5% of its weight and contain considerable amounts of carbohydrates, proteins, oil, dietary fiber and ash [22, 23], as well as bioactive substances such as polyphenols, flavonoids, and carotenoids [24]. Polyphenols possess antioxidant, antimutagenic and antitumor properties, flavonoids have free radical scavenging capacity, anti-inflammatory, antiallergic, antiviral and anticancer properties, while carotenoids contribute to the health system due to cell communication, antioxidant activity, immune function, provitamin A activity and skin protection against ultraviolet damage [17]. Another important contribution of noni seeds is its oil that contains fatty acids such as lauric, linoleic, oleic, palmitoleic, and stearic acids, which have immunostimulant, anti-inflammatory, anticarcinogenic, or hypotensive activities, as well as the antimicrobial activity of its extracts against bacteria and fungi [25]. Also, in a recent study with mice, the gastroprotective effect of a peptide isolated from noni seeds was demonstrated by improving inflammation and oxidative stress [26].

As can be observed, there are several chemical compounds that have been recovered from noni seeds for their possible use as bioactive or antimicrobial agents, however, at the light of our knowledge, to date no study has focused on the recovery of proteins in the form of protein concentrate or isolate for its possible use as a food ingredient. Therefore, the objectives of this research were: a) to prepare a protein isolate from noni seeds by means of alkaline extraction and isoelectric precipitation, and b) to evaluate its physicochemical, functional, and structural characteristics for its possible application in the food industry.

Material and Methods

Raw Material and Chemicals

By-products from the processing of the noni juice (BNJ) from fruit grown in the town of Valle de Banderas, Nayarit, México were used to obtain the seeds, which were transported to the Centro de Tecnología de Alimentos of the Universidad Autónoma de Nayarit and refrigerated at 4 °C for later usage. Noni seeds (NS) were manually retrieved from the BNJ, washed under running water until all impurities were removed, and excess water was drained into a plastic strainer. Following, the washed clean NS were dehydrated in an oven at 40°C for 48 h (Fig. 1A). All chemicals used in this study were reagent grade and purchased from J.T. Baker and Sigma-Aldrich (Mexico City, Mexico).

Determination of Physical Properties of Noni Seeds (NS)

NS geometrical properties of equivalent diameter (D_{eq}), arithmetic mean diameter (D_{ma}), and sphericity (S_{phe}) were calculated from three dimensional characteristics (mm): length (L), width (W), and thickness (T), which were measured by a digital vernier caliper (accuracy 0.001) on 100 seeds, with the following methods and Eqs. 1–3 reported by Ortiz-Hernandez et al. [27].

$$D_{eq} (mm) = \sqrt[3]{L \times W \times T}$$

1

$$D_{ma} (mm) = \frac{L + W + T}{3}$$

2

$$S_{phe} (\%) = \left[\frac{D_{eq}}{L} \right] \times 100$$

3

Gravimetric and frictional properties of NS were determined according to methods and Eqs. [6](#) reported by Anandakumar et al. [28].

Weight of one hundred NS was measured by weighing of the seeds counted by manually, using digital precision balance with 0.001 g accuracy.

Bulk density (ρ) was determined by pouring the NS into a 25 ml cylinder, then weighing the NS, and its value was calculated using Eq. 4.

$$\rho (g/ml) = \frac{\text{Weight of the NS}}{\text{Volume of the NS}}$$

4

True density (ρ_t) was determined using the toluene displacement method using a 25 ml cylinder, where the 5 g of NS were immersed into toluene. The volume of displaced toluene was recorded from the cylinder. The ratio of weight of the seeds and the volume of the displaced toluene gave the ρ_t of the NS.

The porosity (P_o) of the NS was calculated using the Eq. 5.

$$P_o (\%) = \frac{\rho_t - \rho}{\rho_t} \times 100$$

5

Angle of repose (A_r) was defined by using the steel funnel consisting of a cone at the top and a cylinder at the bottom, applying the experimental stand and calculated by the Eq. 6.

$$A_r (\text{degrees}) = \tan^{-1} \left[\frac{2h}{D} \right]$$

6

where h = height of the heap, and D = diameter of the heap.

Preparation of the Deoiled Noni Seed Flour (DNSF)

The previously dehydrated NS were pulverized in a Nutribullet home food processor model NB101B (Ningbo Bestwin Industrial Co., Ltd., China) to obtain the noni seed flour (NSF). Next, portions of 100 g of NSF were deoiled using 1,000 ml ethylic ether and magnetic stirring at 300 rpm and 25°C by means of 4 subsequent extractions where the exhausted solvent was replaced with new solvent. Finally, the NSF obtained from the last extraction was desolventized in a fume extraction hood for 8 h on a stainless-steel tray and the product was named DNSF (Fig. 1B)

Elaboration of Noni Seed Protein Concentrate (NSPC)

NSPC was elaborated according to the procedure of Flores-Jiménez et al. [12] with some modifications. Initially, protein solubility profile against pH (2–12) was determined to define the pH values for the maximum protein extraction and the isoelectric precipitation. 50 g of DNSF were added to 1,000 ml distilled water adjusting the pH with NaOH 0.1 N at the value previously defined for the maximum protein extraction. The slurry was subjected to magnetic stirring at 300 rpm and 25°C for 30 min and then centrifugated at 10,000 rpm for 10 min. Next, the supernatant was adjusted at the pH value of lower protein extraction previously determined (isoelectric point) with HCl 0.1 N, the obtained slurry stirred for 30 min at 20°C and then centrifugated at 10,000 rpm for 10 min. Right away, the precipitate proceeding of the previous stage was treated with ethanol (95%) in a ratio 1:30 (v/v) during 30 min with magnetic stirring. Finally, the protein precipitate was recovered by centrifugation at 10,000 rpm for 10 min, resuspended in distilled water at 1:10 (w/v) ratio, adjusted to pH 7.0 with 0.1 NaOH, and lyophilized into

50 ml plastic tubes in a unit FreeZone model 10 L (Labconco, USA), obtaining a powder called NSPC, which was stored in hermetically closed glass jars for further analysis.

Physicochemical Analyses

The contents of protein (N x6.25), lipids, ash, crude fiber, and moisture were determined following the Association Official of Analytical Chemists International [29] methods. The carbohydrate content was calculated by difference from 100 and the sum of the contents of the components previous. Color was measured with a Minolta CR-400 color meter (Minolta Ltd, Co., Tokyo, Japan) using the CIELAB color scale, where L^* = lightness, $+a^*$ = redness, $-a^*$ = greenness, $+b^*$ = yellowness and $-b^*$ = blueness. A white standard tile, where $L_s^* = 92.9$, $a_s^* = -5.29$, and $b_{ss}^* = 8.76$ was used to calibrate the color meter. By means of Eq. 7, the total color difference (ΔE) was calculated:

$$\Delta E = \sqrt{(L_s^* - L^*)^2 + (a_s^* - a^*)^2 + (b_{ss}^* - b^*)^2}$$

7

Water activity (a_w) was measured with an AquaLab 4TEV (Decagon Devices Inc., Pullman, WA, USA) equip. Bulk density (ρ) was determined according to procedure reported by Sahin et al. [30], relating the mass of the sample contained in a 10-mL mark of a tared graduated cylinder and expressed as g/ml.

Functional Analysis

Protein solubility was quantified following the method of Wang et al. [31], in aqueous suspensions of NSPC (1%) at pH values of 2, 4, 6, 8, 10 and 12 (adjusted with 0.1 NaOH or HCl) prepared by magnetic stirring for 30 min, measuring the protein recovered in the supernatant after centrifuging at 10,000 rpm for 10 min, with respect to the NSPC protein content and expressed as a percentage.

Both water-holding capacity ($WHCa$) and oil-holding capacity ($OHCa$) were determined using the method of Li et al. [32], in tubes with 2.0 g of NSPC added with 5 g of distilled water or canola oil, respectively. Tubes were shaken in a vortex for 30 s, rested for 30 min, centrifuged at 5,000 rpm for 20 min, and decanted from the free water or oil, expressing $WHCa$ and $OHCa$ as g H₂O or oil per g protein, respectively.

Foaming properties were evaluated as foaming capacity (FoC) and foaming stability (FoS) according to the methods reported by Ma et al. [33] with light modifications. 10 ml of a 1% (w/v) aqueous dispersion of the NSPC was mixed in a 50 ml tube with a T-25 Ultra-Turrax homogenizer (IKA Instrument, Germany) at 10,000 rpm for 2 min, transferring the product to a 100 ml measuring cylinder to determine the volume after blending. FoC and FoS were calculated with the Eqs. 8 and 9, respectively, where V_1 and V_2 are the initial foam volumes at 0 time and after 30 min.

$$FoC (\%) = \frac{V_1}{10} \times 100$$

8

$$FoS (\%) = \frac{V_2}{V_1} \times 100$$

9

Emulsifying properties represented by emulsifying activity (A_{em}) and emulsion stability (S_{em}) were defined using the Ulloa et al. [34] methods. In a 50 ml tube, to 10 ml of a 1% (w/v) NSPC aqueous suspension was added 10 ml of canola oil, which was mixed with a T-25 Ultra-Turrax homogenizer at 12,000 rpm for 1 min and then centrifuged at 1,200 rpm for 5 min for determination of emulsion layer volume. For emulsion stability, the tube was heated in a water bath at 80°C for 30 min, cooled to 25°C in running water and centrifuged as above. The values of A_{em} and S_{em} were calculated with the Eqs. 10 and 11, respectively, where V_{em} = emulsifying layer volume, V_t =total volume of tube contents, and V_{emf} = emulsion volume after heating and centrifugation.

$$A_{em} (\%) = \frac{V_{em}}{V_t} \times 100$$

10

$$S_{em} (\%) = \frac{V_{em}}{V_{emf}} \times 100$$

11

The gelling properties of the NSPC were evaluated as the least gelation concentration ($LGeC$) according to Benelhadj et al. [35] method with minor modifications. The NSPC samples were mixed with 5 ml of distilled water in centrifuge tubes to obtain concentrations of 1, 2, 3, 4, 5 and 6% (w/v). Centrifuge tubes were heated for 1 h in a boiling water bath, quenched under running tap water, and then chilled for 2 h in a refrigerator at 4°C. $LGeC$ was considered as the lowest concentration at which the sample in the inverted tube did not fall or slip.

In vitro digestibility ($InVD$) was determined following the reported method by Wang et al. [31] with light modifications. 1.5 ml of a multi-enzymatic suspension (0.05 mg pepsin/ml + 1 mg pancreatin/ml, at pH 8.0) was added to a protein suspension prepared with 62.5 mg of NSPC in 10 ml of water (adjusted to pH 8.0) and maintained at 37°C. At enzymatic reaction time of 10 min the pH value was measured and used to calculate the $InVD$ with the Eq. 12.

$$InVD (\%) = 210.464 - 10.103 (pH_{at10min})$$

12

Structure Analysis

The NSPC microstructure was observed at 200x with a SNE-3200 scanning electron microscopy (SEC Co., LTD, Suwon, South Korea), at an accelerating voltage of 20 kV, after coating the sample with a thin layer

of gold using an MCM-100 ion spray coater.

Proteins fractions based on their solubility from DNSF and NSPC were consecutively extracted in water, 0.5M NaCl, 0.1M NaOH, and 70% ethanol at 25 °C, according to the Osborne methodology as reported by Amza et al. [36]. The total protein contents of prolamin, glutelin, globulin, and albumin fractions were determined by Kjeldahl method ($N \times 6.25$).

Statistical Analysis

The results of the physicochemical, functional, and structure analyses were performed in triplicate and stated as the average $\pm$ standard deviation. When three treatments were involved, the results were subjected to a one-way analysis of variance, using Tukey's test for multiple comparison of means at a significance level $p < 0.05$, while for the comparison of two treatments the student's t test was applied ($p < 0.05$). Data processing was carried out by means of IBM SPSS Statistics 20 software (IBM, New York, United States of America).

Results and Discussion

NS Physical Properties

Due to the importance as well as the growing interest in the valorization of fruit processing by-products, as is the case of its seeds, it is necessary to determine its physical properties, among which the geometric, gravimetric, and frictional properties stand out [37]. The information on these physical properties is relevant to evaluate their productive viability, as well as the technoeconomic of seed handling and storage, since they are directly related to the design of the equipment for their handling, husking, separation, drying, aeration, storage, and post processing [38].

Table 1 shows the physical properties of the NS. The mean values for L , W , and T (expressed as mm) for the NS were 9.127 ± 1.000 , 4.320 ± 0.721 , and 2.090 ± 0.407 , respectively, resulting smaller than 10.8 ± 0.63 , 6.8 ± 0.43 , and 2.3 ± 0.25 for watermelon seeds [39], but greater than 7.79 ± 0.50 , 3.34 ± 0.30 , and 1.20 ± 0.19 for melon seeds [40]. The D_{eq} and D_{ma} parameters are useful in evaluating the size of the opening of the grading equipment for seeds moving in the air current of the turbulent zone [38], being the values of such parameters for NS of 4.327 ± 0.485 mm and 5.182 ± 0.510 mm, respectively. The S_{phe} of the seeds is an important criterion to know the tendencies of the free flow of their mass in cleaning and other operations, and this represents their roundness, that is, the indirect expression in percentage terms of how close their shape is to that of the sphere [41]. In the case of the NS, the S_{phe} was $47.655 \pm 4.935\%$, which is in the range of values of $36.85 \pm 1.80\%$ for melon seeds [40] and $55.43 \pm 0.53\%$ for yellow passion fruit seeds [42].

Table 1
Physical properties of the noni seeds

Properties	Value
<i>Geometrical</i>	
Lenght (L , mm)	9.127 ± 1.000
Width (W , mm)	4.320 ± 0.721
Tickness (T , mm)	2.090 ± 0.407
Equivalent diameter (D_{eq} , mm)	4.327 ± 0.485
Arithmetic mean diameter (D_{ma} , mm)	5.182 ± 0.510
Sphericity (S_{phe} %)	47.655 ± 4.935
<i>Gravimetic and frictional</i>	
Weight of 100 seeds (W_{100s} , g)	2.137 ± 0.054
Bulk density (ρ , g/ml)	0.276 ± 0.003
True density (ρ_t , g/ml)	0.608 ± 0.011
Porosity (P_o %)	54.605 ± 0.331
Angle of repose (A_r , degrees)	31.995 ± 0.158
Data presented as mean values $\pm$ standard deviation. For geometrical properties $n = 100$. For gravimetrical and frictional properties $n = 3$	

The definition of average values of ρ , ρ_t and P_o is important to design and optimize the size of seed containers, as well as to choose the type of material and volume for the hoppers in seed packaging equipment [28]. ρ , ρ_t and P_o for NS were 0.276 ± 0.003 g/ml, 0.608 ± 0.011 g/ml and $54.605 \pm 0.331\%$, respectively, in contrast to 0.146 ± 0.001 g/ml, 0.861 ± 0.001 g/ml, and $51.681 \pm 1.452\%$ for watermelon seeds variety Sarakhsy [43], and 0.42 ± 0.006 g/ml, 0.65 ± 0.021 g/ml, and $45.4 \pm 0.54\%$ for black *Jatropha curcas* seeds [44]. A_r depends on the shape, size and surface condition of the seeds [45] and is a very important parameter in the characterization of their flow/sliding properties, which is related to friction between seeds or resistance to movement between seeds [41]. A_r (as degree) for NS was 31.995 ± 0.158 in comparison with 29.51 ± 0.18 for pomegranate seeds [46], 32.9 ± 1.1 for black *Jatropha curcas* seeds [44], and 32.38 ± 0.47 for watermelon seeds variety Red [43].

Conditions of NSPC Preparation

Figure 2A shows the effect of pH on the protein extraction from DNSF. Maximum protein extraction of 90.8% was at pH 12, while the minimum protein extraction of 13.0% was at pH 3 (isoelectric point).

Therefore, such pH values were chosen for the preparation of the NSPC by alkaline extraction and isoelectric precipitation. The pH values for protein extraction and isoelectric precipitation from mango [11] and orange [13] deoiled seed flours were 12 and 4.5 and 11 and 5, respectively, obtaining maximal and minimal protein extractions of 53.4% and 6.9%, and 90.5% and 30.7%.

Composition Chemical of NSCI

Table 2 shows the chemical composition of NSCI, as well as that of NSF and DNSF. The protein content was raised 10.4-fold and 10.14-fold from NSF and DNSF, respectively, to obtain the protein content of 76.59% for the NSPC. Such protein concentration of the NSPC was mainly due to the reduction of the oil content in the DNSF obtained from the NSF, in addition to the decrease of the fiber and carbohydrate contents, in the alkaline extraction and isoelectric precipitation steps during the preparation of the NSPC from the DNSF. Lower protein contents than the obtained for the NSPC of this study have been reported for other protein concentrates from fruit seeds as mango (75.0%) [11], guamuchil (75.17–76.40%)[47], and pomegranate (45.8%)[48]. On the other hand, protein content values similar to or higher than those obtained for the NSPC have also been reported for other protein concentrates from jackfruit (76.89%) [49] and tomato (80.37%) [50] seeds.

Table 2
Chemical composition of the noni seed flour (NSF), deoiled noni seed flour (DNSF), and noni seed protein concentrate (DNSF)

Component	Content (%)		
	NSF	DNSF	NSPC
Protein (Nx6.25)	7.35 ± 0.04 ^a	10.72 ± 0.02 ^b	76.59 ± 0.30 ^c
Moisture	6.94 ± 0.05 ^a	6.73 ± 0.05 ^b	7.67 ± 0.07 ^c
Ethereum extract	14.35 ± 0.81 ^a	0.36 ± 0.05 ^b	6.99 ± 0.05 ^c
Ash	1.30 ± 0.06 ^a	1.11 ± 0.05 ^b	8.44 ± 0.12 ^c
Crude fiber	35.70 ± 0.80 ^a	41.40 ± 1.00 ^b	0.00 ^c
Carbohydrates ^y	34.36 ± 0.50 ^a	40.45 ± 0.32 ^b	0.29 ± 0.47 ^c
Data presented as mean values ± standard deviation. Different superscripts within the same row are significantly different (p < 0.05). ^y Calculated by difference			

The physicochemical properties of the NSPC are shown in Table 3. The color of protein materials influences the appearance and acceptance of food products when used as ingredients, since this property is what initially allows contact with consumers [51]. *L**, *a**, *b** and ΔE values for the NSPC were 55.32 ± 0.08, 4.60 ± 0.02, 13.09 ± 0.01, and 61.46 ± 0.11, respectively, whose aspect can be appreciated in Fig. 3A. For the guamuchil proteins [12], the values of *L** (50.99 ± 0.44) and *a** (1.93 ± 0.13) were lower than those

of the NSPC of this study, but in the case of b^* (27.82 ± 0.07) its value was higher than that of the NS proteins. In another study with proteins of bitter melon seeds were obtained values de $L^* = 74.96 \pm 0.19$, $a^* = 1.49 \pm 0.04$, and $b^* = 18.19 \pm 0.09$ [51], being L^* and b^* higher than the values for the NSPC, but the a^* value was lower than the obtained for the noni seed proteins isolated of this investigation.

Table 3
Physicochemical properties of the noni seed protein concentrate

Parámetro	Value
Color	
Lightness (L^*)	55.32 ± 0.08
Redness (a^*)	4.60 ± 0.02
Yellowness (b^*)	13.09 ± 0.01
Total color difference (ΔE)	61.46 ± 0.11
Water activity (a_w)	0.397 ± 0.001
Bulk density (pb, cm ³)	0.056 ± 0.01
Data presented as mean values $\pm$ standard deviation (n = 3)	

In food powders, a_w is an important parameter associated with their chemical and microbiological stability during storage [52]. The a_w value of the NSPC was 0.397 ± 0.001 , which is below 0.66 that is the cutoff value to prevent microbial growth [53]. The a_w value of the NSPC was above those obtained for proteins from guamuchil (0.209 ± 0.002) and orange (0.370 ± 0.001) seeds, according to the reported by Flores-Jimenez et al. [12] and Rosas Ulloa et al. [13], respectively. ρ is a fundamental property to characterize powder foods, since has economic and functional implications, affecting the behavior of dry mixes and the volume required by the powder for packaging; this property depends on interrelated factors such as particle size, the number of contact points, and the intensity of the attractive forces between particles [54]. The ρ value of the NSPC was extraordinarily low (0.056 ± 0.01 g/ml) in comparison with other proteins obtained from fruit seeds by alkaline extraction and isoelectric precipitation, as sugar baby watermelon (0.51 ± 0.19 g/ml), fruit passion (0.21 ± 0.01 g/ml), guamuchil (0.408 ± 0.01 g/ml), and orange (0.282 ± 0.005 g/ml), according to the reported by Wani et al. [55], Espinosa-Murillo et al. [10], Flores Jiménez et al. [12], and Rosas Ulloa et al. [13], respectively.

Functional Properties of NSPC

Solubility is a key factor of the proteins for its utilization as ingredient in foods since influences on the other functional properties [31]. The solubility profile of the NSPC protein as a function of pH is shown in Fig. 2B, where it can be seen that the lowest protein solubility of 13.32% occurred at pH 3, increasing progressively with the increase in pH until maximum value of 72.0% at pH 12. This same tendency about

the increase of the protein solubility with the rise of pH from isoelectric point has been observed for other proteins from seed fruits as fruit passion [10], orange [13], and jackfruit [56].

When protein concentrates or isolates are used as ingredients in food formulations, their functional properties are very important since they favor the quality of the products and the profitability of the processes [57]. Table 4 shows the functional properties of the NSPC. The *WHCa* of the NSPC was 4.36 ± 0.24 g H₂O/g protein, resulting in 43.9% (3.03 ± 0.06 g H₂O/g protein) superior to that obtained from orange seeds [13] and 56.8% (2.78 g H₂O/g protein) to that produced from jackfruit seeds [49]. *WHCa* is a decisive parameter in the determination of the techno functionality of the proteins, which is associated with the water retention, swelling, and gelation, also affecting the food product formulation and the hydration with dehydrated ingredients [58].

Table 4
Functional properties of noni seed protein concentrate

Functional properties	Value
Water holding capacity (<i>WHCa</i> , g H ₂ O/g protein)	4.36 ± 0.24
Oil holding capacity (<i>OHCa</i> , g oil/g protein)	11.69 ± 0.50
Emulsifying activity (<i>A_{em}</i> , %)	29.20 ± 0.79
Emulsion stability (<i>S_{em}</i> , %)	50.00 ± 1.00
Foaming capacity (<i>FoC</i> , %)	180.33 ± 0.57
Foaming stability (<i>FoS</i> , %)	94.60 ± 0.34
Least gelling concentration (<i>LGeC</i> , %)	4.00 ± 0.00
<i>In vitro</i> digestibility (<i>InVD</i> , %)	78.51 ± 0.39
Data presented as mean values $\pm$ standard deviation (n = 3)	

The *OHCa* of the NSPC was 11.69 ± 0.50 g oil/g protein (Table 4), value that was extraordinarily higher than 1.26 ± 0.07 g oil/g protein, 1.40 g oil/g protein, and 3.2 ± 0.1 g oil/g protein for proteins from guamuchil [12], tamarind [59], and mango [11] seeds, respectively. *OHCa* is a relevant characteristic of the proteins when are used in bread, soups, and meat extensors, since that high values in this property improves the flavor retention, as well as the soft mouthfeel [11].

Emulsifying properties of the NSPC are presented in Table 4. The *A_{em}* and *S_{em}* of the NSPC were $29.20 \pm 0.79\%$ and $50.00 \pm 1.00\%$, respectively, in comparison with the values of $47.5 \pm 1.8\%$ (at pH 7) and $47.6 \pm 1.9\%$ (at pH 7) for proteins from fruit passion [10] and $62.34 \pm 0.29\%$ and $67.65 \pm 0.34\%$ tamarind [59] seeds. The emulsifying properties of proteins are very important for many food applications, such as frozen desserts, salad dressings, ground meats, mayonnaise, cake batters, milks, and coffee whiteners [9].

Proteins are the main used biopolymers for the formation and stabilization of aerated structure products in food industry, whether liquids (beer, sparkling wine, carbonated soft drinks, coffee beverages), semi-solids (ice cream, whipped cream, aerated desserts) and solids (bread, cakes, breakfast cereals, aerated chocolate bars) [60]. *FoC* and *FoS* of the NSPC were $180.33 \pm 0.57\%$ and $94.60 \pm 0.34\%$, respectively (Table 4), which were higher than $59.10 \pm 1.01\%$ and $86.34 \pm 5.01\%$ for proteins from soursop seeds [61], and 58.5% (at pH 7.5) and 26.0% (at pH 7.5) for proteins from jackfruit seeds [49].

Proteins as concentrates or isolates are widely used as gelling agents to improve the texture and water retention capacity of food products, for example, emulsified meat products such as salami and sausages [11]. *LGeC* of the NSPC was 4.0% (Table 4), being same that the obtained at pH 7 for proteins from fruit passion seeds [10], but lower that 14% for proteins from orange seeds [13], and 20% for proteins from guamuchil seeds [12].

Digestibility is one of the important criteria for determining protein quality, and those with high *InVDi* values are considered to be of high quality, since their amino acids can be incorporated into body protein synthesis after digestion. The *InVDi* of the NSPC was $78.51 \pm 0.39\%$, which is in the range of the values obtained of 71.3% for tamarind seed proteins [62] and 88% for watermelon seed proteins [63].

Structure of NSPC

The microstructure of the NSPC by scanning electron microscopy is shown in Fig. 3B. As can be seen, the NSPC powder particles exhibited irregular, flat, and disordered structures with sharp edges, somewhat similar to other proteins obtained by alkaline extraction, isoelectric precipitation, and freeze-drying from passion fruit [10], orange [13] and jackfruit [9] seeds.

The protein fractional composition of the NSPC is shown in Table 5. A significant difference ($p < 0.05$) in the protein fractional composition between the DNSF and the NSPC was observed, due to the protein isolation by alkaline extraction and isoelectric precipitation. The contents of albumins and glutelins were increased by 1,011.5%. and 17.2% in the NSPC, respectively, in comparison with the DNSF, while the contents of globulins and prolamins were reduced in 35.8% and 81.8%. As in the NSPC of this study, it has also been reported that the predominant fraction for the proteins obtained from orange [13] and guamuchil [12] seeds was glutelins.

Table 5

Protein fractional composition of the deolied noni seed flour (DNSF) and noni seed protein concentrate (NSPC)

Source	Protein fractions (%)			
	Albumins	Globulins	Prolamins	Glutelins
DNSF	2.17 ± 0.02 ^a	8.47 ± 0.02 ^a	34.92 ± 0.96 ^a	55.12 ± 0.53 ^a
NSPC	24.12 ± 0.56 ^b	5.44 ± 0.13 ^b	6.34 ± 0.80 ^b	64.62 ± 0.63 ^b
Data presented as mean values ± standard deviation (n = 3). Different superscripts within the same row are significantly different (p < 0.05)				

Conclusions

Proteins from NS were recovered successfully by alkaline extraction and isoelectric precipitation, in form of NSPC with a protein purity of 76.59%. The NSPC is an alternative for the valorization of noni seeds proceeding from juice industry, which previously had not been studied as source of proteins for applications in foods. The results obtained in this study about the physicochemical, functional, and structural properties of the NSPC suggest that said powder could be used as an ingredient in food for human consumption, to strengthen or enhance its sensory characteristics. Specific applications of the NSPC as ingredient in the different types of foods or for the development of new food products should be tested in further studies.

Declarations

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Author Contributions. JAHR: Investigation, Methodology, Writing – original draft. JAU: Investigation, Writing – original draft, Writing – review & editing, Conceptualization, Supervision, Data curation, Funding acquisition. PRU: Software, Supervision, Validation. BEUR: Conceptualization, Supervision, Visualization.

Data Availability. Data is available on reasonable request.

Conflicts of Interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the study reported in this article.

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Figures

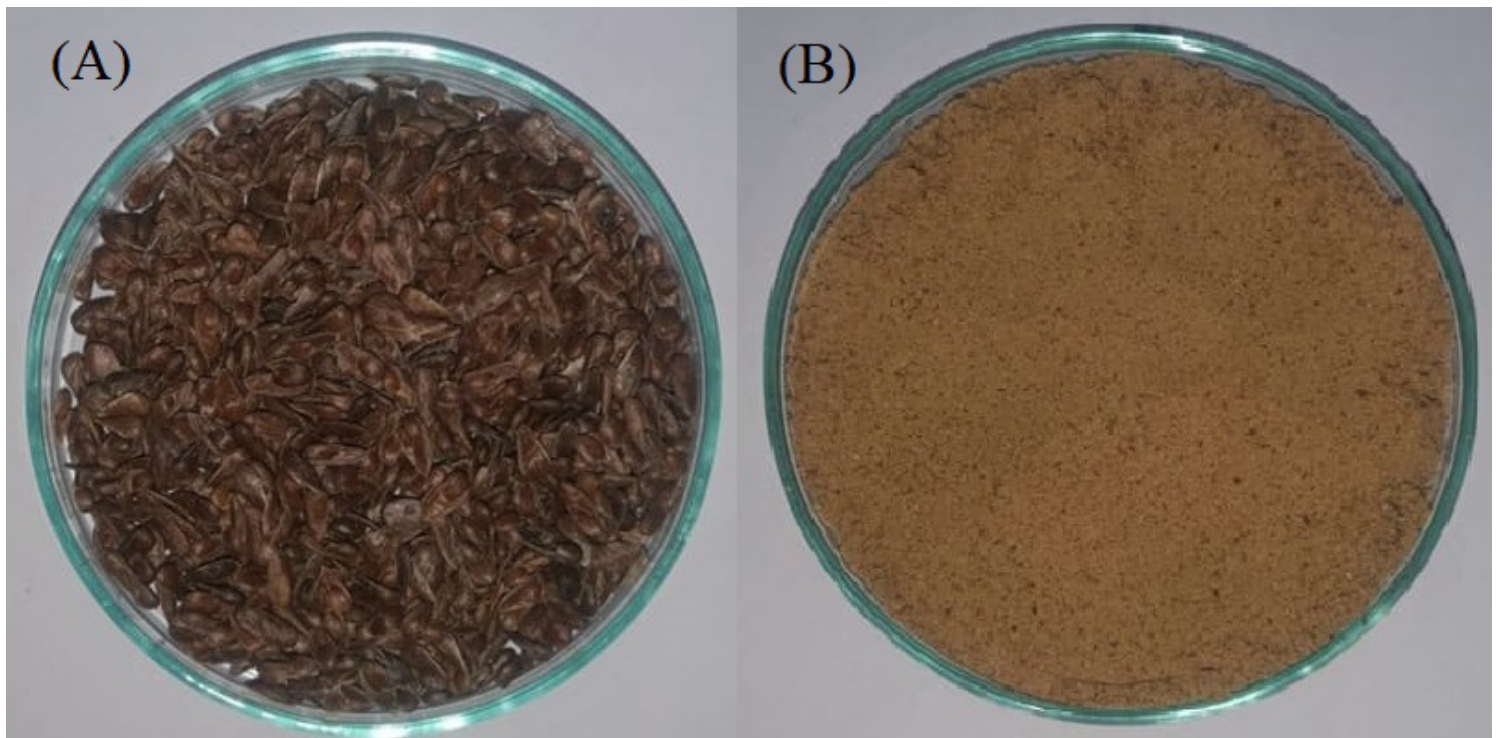


Figure 1

Aspect shown by: (A) noni seeds and (B) deoiled noni seed flour

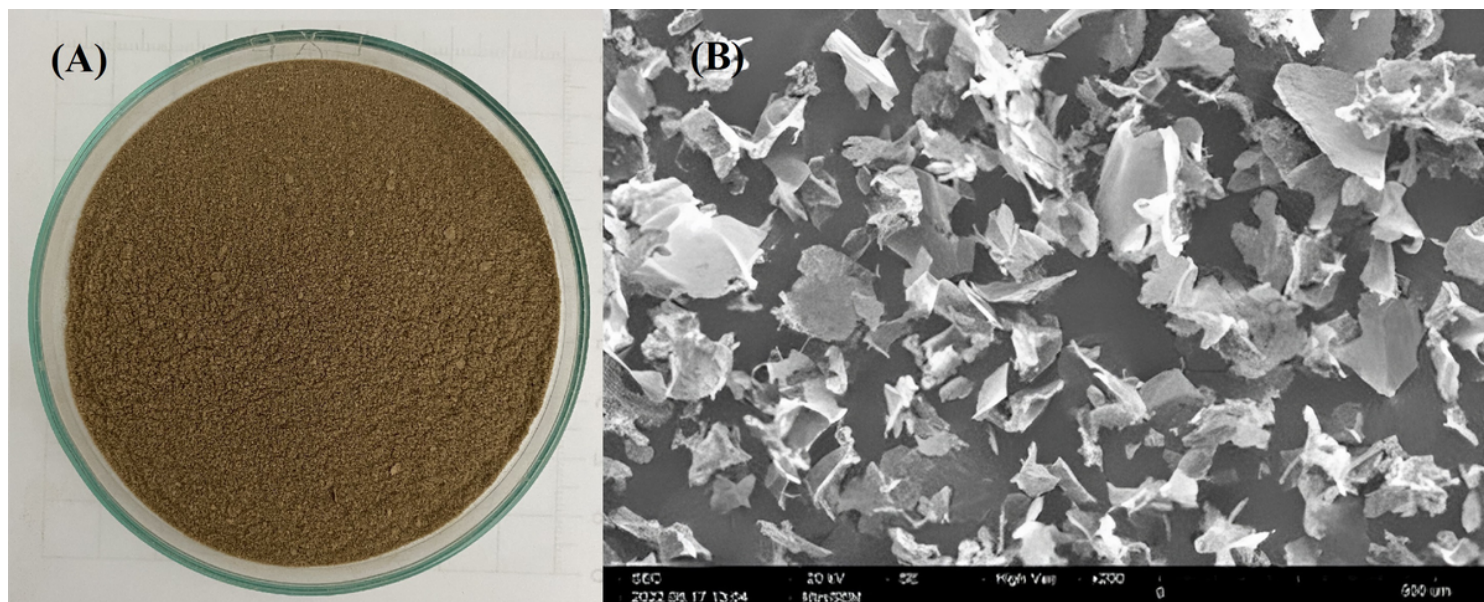


Figure 2

Effect of pH on: (A) protein extraction from deoiled noni seed flour and (B) protein solubility of the noni seed protein isolate. Same letters indicate non-significant differences ($p > 0.05$)

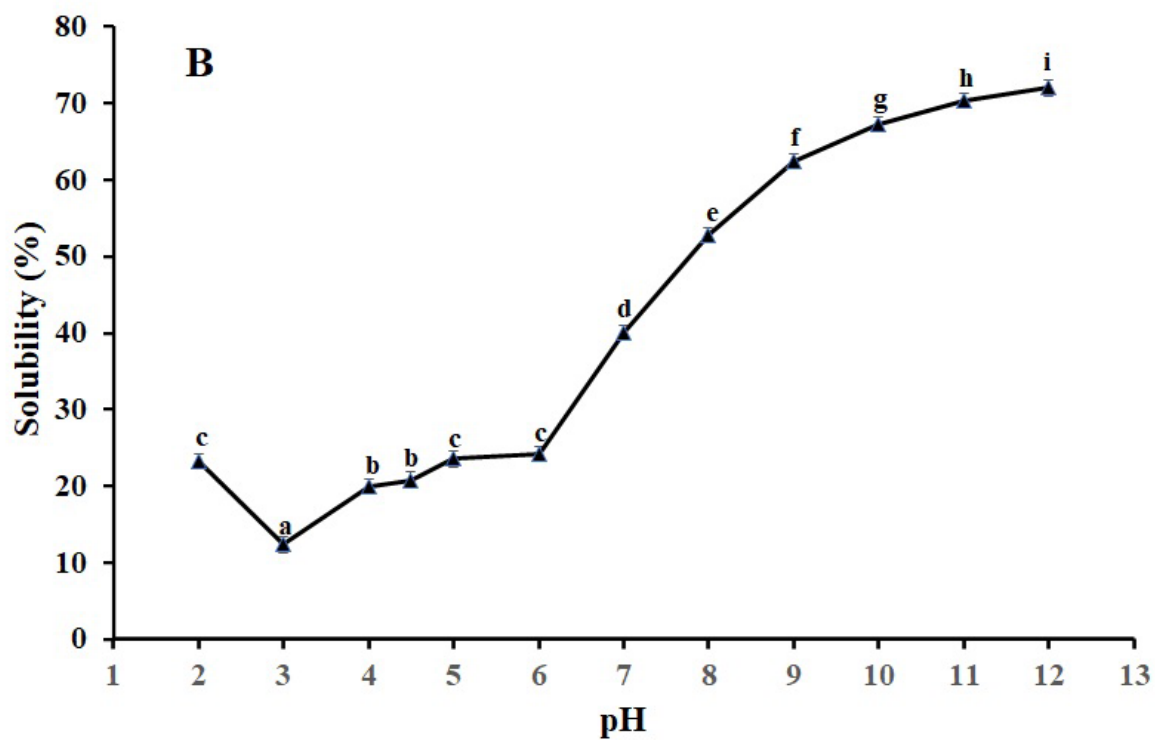
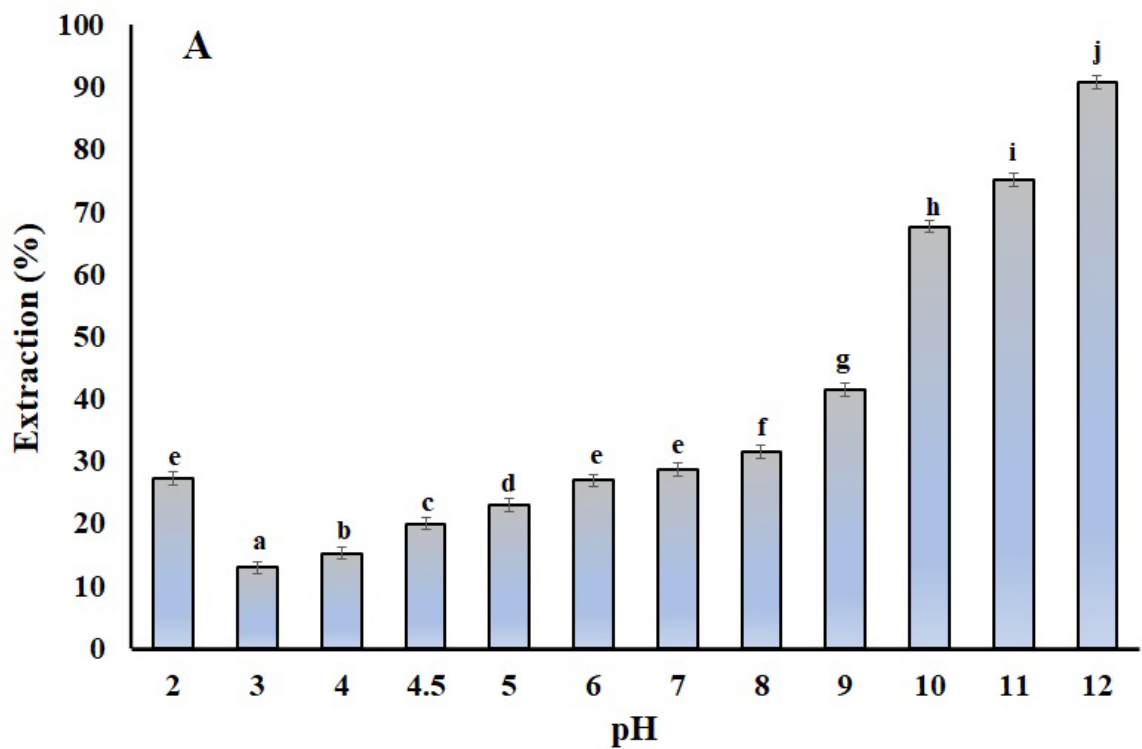


Figure 3

Aspect shown by: (A) noni seed protein concentrate and (B) microstructure of noni seed protein concentrate by scanning electronic microscopy (x 200)

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