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Research article

Evaluation of the functional properties of a protein isolate from *Arthrospira maxima* and its application in a meat sausage

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ABSTRACT

Arthrospira maxima is a microalga that has been collected in Lake Texcoco in the Valley of Mexico since pre-Hispanic times and has been a traditional food source due to its high biomass production and protein content (50–60 %), making it promising for protein extraction. In this context, a protein isolate was obtained from powdered biomass of *Arthrospira maxima* (PbAm) by alkaline solubilization (pH 11) and isoelectric precipitation (pH 4.2). *Arthrospira maxima* protein isolate (AmPI) presented higher protein content (82.58 %) and total amino acids compared to PbAm. Functional properties of AmPI were evaluated in comparison with PbAm and soy protein isolate (SPI). Protein extraction resulted in a significant increase in protein solubility (PS) and foaming capacity (FC) of up to 87.78 % and 238.10 %, respectively. Emulsifying capacity (EC) of AmPI was superior to that of PbAm and SPI in pH range 5–7. Inclusion of AmPI as a partial substitute for SPI in the formulation of meat sausages was evaluated by implementing four treatments: T1 (15 % AmPI, 85 % SPI), T2 (10 % AmPI, 90 % SPI), T3 (5 % AmPI, 95 % SPI) and T4 (0 % AmPI, 100 % SPI). Although the texture attributes remained unchanged, a significant reduction in color parameters was observed as the concentration of AmPI increased. An inclusion of 15 % AmPI significantly enhanced the nutritional quality of meat sausages. Results highlight the excellent properties of AmPI, confirming *Arthrospira maxima* as a promising protein source in the food industry.

1. Introduction

Plant-based products rich in proteins, primarily obtained from soybeans, peas, and wheat, are widely used in the market, particularly in the form of protein concentrates and isolates, due to their high demand in dietary supplementation [1]. In the meat industry, soy proteins are employed for their functional properties, nutritional value, availability, and ability to mimic meat characteristics [2]. However, concerns arise regarding obtaining this protein source.

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Socioeconomic effects have been recorded, including an economic growth concentrated solely in the regions where soy is produced. Additionally, severe environmental impacts stemming from the increase in its production have been identified [3]. These include deforestation, soil degradation, depletion of water resources, loss of biodiversity, and greenhouse gas emissions, primarily carbon dioxide (CO2) [1]. Therefore, actively researching more sustainable alternative protein sources, including plants, fungi (mycoproteins), insects, bacteria, algae, and microalgae, is underway [4].

Microalgae stand out as promising protein sources for partial or total substitution of plant proteins in food applications, due to their rapid cell growth, high surface productivity, and superior photosynthetic efficiency. Additionally, they excel in their lower environmental impact, as they require less water, energy, and land space compared to conventional agricultural crops such as soy [5]. The genus *Arthrospira*, commercially known as *Spirulina*, mainly comprises two species of cyanobacteria: *Arthrospira platensis* and *Arthrospira maxima* [6]. *A. maxima* exhibits a predominant distribution in Central America [7]. Mexico, with its favorable climatic conditions, has cultivated *A. maxima* both on a commercial scale and at a small scale [6]. The Texcoco Lake in Mexico is believed to have been the original habitat of *A. maxima*, where it was already being collected as a pre-Hispanic food source in the 16th century [7]. This species is characterized by its biochemical composition, which includes a high protein concentration (50–60 %) with excellent digestibility (80–93 %) and an essential amino acid content constituting 47 % of its total protein [8]. *A. maxima* has been recognized by the Food and Drug Administration (FDA) as an excellent source of protein, with the designation GRAS (Generally Recognized as Safe) [6]. This recognition highlights its potential for obtaining various valuable bioproducts, such as natural colorants and pigments, antioxidant compounds, omega-3-rich oils, carbohydrates, as well as protein concentrates and isolates for dietary supplementation [9].

Previous studies have utilized the alkaline solubilization and isoelectric precipitation method to obtain protein concentrates and isolates from various microalgae, such as *A. platensis* [10], *S. platensis* LEB 52 [8], *Spirulina* sp. LEB 18 [1], and *Nannochloropsis oculata* [11]. These products are characterized by their remarkable protein contents, ranging between 75 and 91 %, and exhibit notable functional properties, particularly in terms of solubility, foaming and emulsifying capacity [1,8]. Therefore, they represent excellent choices for incorporation into meat products.

Research in meat products aims to create healthier and more sustainable sausages without compromising their quality. This is achieved by incorporating non-meat additives such as starches, gums, fibers, and proteins, including those derived from microalgae [12]. The successful incorporation of microalgae biomass, such as *Spirulina* and *Chlorella* [5], and *Chlorella vulgaris* [12], into pork sausages has enhanced their nutritional value. While several microalgae show significant potential as promising protein sources, previous research has primarily focused on *Chlorella vulgaris* and *Arthrospira platensis*. This underscores the importance of exploring the potential of *Arthrospira maxima* as biomass and protein isolate.

In this context, the main objective is to obtain an *Arthrospira maxima* protein isolate (AmPI) through the alkaline solubilization and isoelectric precipitation method, and to characterize its proximal and structural composition. Evaluate its functional properties in comparison with the powdered biomass of *A. maxima* (PbAm) and soy protein isolate (SPI). Additionally, assess the techno-functional suitability of AmPI as a partial substitute for SPI in meat sausages, considering its influence on the nutritional composition, physicochemical properties, and texture of the product.

2. Materials and methods

Fresh biomass of *A. maxima* (FbAm) was obtained from the cultures established outdoors in a mesh-shade greenhouse located in the experimental area of the College of Postgraduates, Córdoba Campus, Veracruz, Mexico. The FbAm was dried in a food dehydrator (Excalibur 2900ECB, USA) at 41 °C for 4 h, ground in a food processor (Nutribullet NB101B, China) and stored until analysis.

For preparation of the meat sausage, the pork meat and back fat were obtained from a local retailer in Córdoba, Veracruz, Mexico, while the ingredients and additives used were purchased from a commercial supply business in the region.

2.1. Obtaining Arthrospira maxima protein isolate

The *A. maxima* protein isolate (AmPI) was obtained using the alkaline solubilization and isoelectric precipitation method, as indicated by Pereira et al. [1] with modifications. Alkaline extraction was performed at pH 11, where the powdered biomass of *A. maxima* (PbAm) was homogenized for 15 min with distilled water at a 1:20 (w/v) ratio using a high-speed turbo blender (Rhino, TURLIC-280, Mexico), and a 1 M NaOH solution was utilized to adjust the pH. It was then subjected to centrifugation (Eppendorf™, Centrifuge 5810/5810R, Mexico) at 4000 rpm, 25 °C, for 15 min. The supernatant was recovered by decantation, and the precipitate was resuspended in distilled water and then subjected to alkaline solubilization once more. The supernatants obtained were adjusted to pH 4.2 using 1 M HCl solution and stirred using a high-speed turbo blender (Rhino, TURLIC-280, Mexico) for 15 min. Subsequently, this solution was centrifuged (EppendorfTM, Centrifuge 5810/5810R, Mexico) at 4000 rpm, 25 °C, for 15 min, and the supernatant was removed. The precipitated protein was dried in a food dehydrator (Excalibur 2900ECB, USA) at 41 °C for 4 h, ground in a food processor (Nutribullet NB101B, China) and stored until analysis. The process was repeated as many times as necessary until enough AmPI was obtained for evaluation and subsequent application.

2.1.1. Yield

The yield of the process for obtaining PbAm was calculated by the weight difference between FbAm and PbAm. The yield of the process for obtaining AmPI was determined by the weight difference between PbAm and AmPI, expressed as a percentage (%).

2.1.2. Proximal composition

The moisture, fat, protein, and ash contents of PbAm and AmPI were determined according to the standard methods proposed by the Association of Official Analytical Chemists [13]. The carbohydrate content was calculated by difference and expressed as a percentage (%).

2.1.3. Structural characterization

A Fourier transform infrared (FTIR) spectrometer (ThermoScientific© iS50, Bruker, Vertex, Wisconsin, USA) was utilized to identify the functional groups present in PbAm, AmPI, and soy protein isolate (SPI), employing attenuated total reflectance (ATR). The spectra were analyzed using Origin 6.1 software (OriginLab Corporation, USA), following the methodology outlined by Blume et al. [14] with modifications.

2.1.4. Amino acid profile

The amino acid profile of PbAm and AmPI was determined by high-performance liquid chromatography (HPLC) using the INS-SM/US-260 method for total amino acids in foods, flours, and protein concentrates.

2.2. Functional properties

2.2.1. Protein solubility

Protein solubility (PS) was determined as established by Morr et al. [15] with modifications. Samples of PbAm, AmPI, and SPI (0.1 g each) were homogenized with 20 mL of distilled water and magnetically stirred (Thermo Fisher Scientific, SP131325Q Cimarec, USA) at 250 rpm for 10 min at 25 °C. The pH of the solutions was adjusted to various values (pH 1, 2, 3, 4, 5, 7, 9, 11, 12, 13) using either 0.1 M HCl or 0.1 M NaOH. Samples were transferred to 20 mL Falcon tubes and centrifuged (Eppendorf™, Centrifuge 5810/5810R, Mexico) at 4000 rpm for 15 min at 25 °C. A 600 μl aliquot was taken from the supernatant to quantify the soluble protein content. The control was prepared under the same conditions, without adjusting the pH or centrifuging. Soluble protein content (g L^{-1}) of the supernatant of the samples and the control was determined according to the method established by Lowry et al. [16]. PS was expressed as a percentage according to Eq. (1).

$$\text{PS}\ (\%) = \frac{\text{Soluble protein of the supernatant}}{\text{Soluble protein of the control}} \times 100 \tag{1}$$

A bovine serum albumin (BSA) calibration curve (1.5 g L^{-1}, purity >96 %) was used with the following linear equation: $y = 0.204545 + 0.001008x$; $R^2 = 0.98$. Absorbances were measured at 750 nm in a UV–Visible spectrophotometer (Jasco Inc., V-730, Tokyo, Japan).

2.2.2. Water absorption capacity

Water absorption capacity (WAC) was determined using the method described by Köhn et al. [17] with modifications. PbAm, AmPI, and SPI samples (0.3 g) were homogenized with 10 mL of distilled water. The solution was adjusted to different pH values (1, 2, 3, 4, 5, 7, 9, 11, 12, 13) with 0.1 M HCl or 0.1 M NaOH and vortexed (IKA®, VORTEX 2, USA) at 25 °C for 10 min. Samples were allowed to stand for 30 min and centrifuged (Eppendorf™, Centrifuge 5810/5810R, Mexico) at 4000 rpm for 15 min at 25 °C. The supernatant was removed by decanting, and the tubes were dried in a forced-air oven for 25 min at 50 °C. WAC was calculated with Eq. (2) and was expressed as a percentage.

$$\text{WAC}\ (\%) = \frac{\text{Weight of water absorbed}}{\text{Initial weight of the sample}} \times 100 \tag{2}$$

2.2.3. Emulsifying capacity and emulsifying stability

Emulsifying capacity (EC) and emulsifying stability (ES) were determined using the method described by Yasumatsu et al. [18] with some modifications. PbAm, AmPI, and SPI samples (0.5 g) were homogenized with 50 mL of distilled water under constant stirring (Thermo Fisher Scientific, SP131325Q Cimarec, USA) at 500 rpm for 30 min at 25 °C. The solutions were adjusted to different pH values (1, 2, 3, 4, 5, 7, 9, 11, 12, 13) with 0.1 M HCl or 0.1 M NaOH. A 50 mL aliquot of neat canola oil was slowly added to the solutions with constant stirring using a high-speed turbo blender (Rhino, TURLIC-280, Mexico) at 20 000 rpm for 2 min until the oil-in-water emulsion was formed. Fixed emulsion volumes (40 mL; total volume) were poured into centrifuge tubes (50 mL) and centrifuged (Eppendorf™, Centrifuge 5810/5810R, Mexico) at 4000 rpm for 5 min. The volume of the emulsified fraction was noted, and EC was calculated according to Eq. (3).

$$\text{EC} \Big/ \text{ES}\ (\%) = \frac{\text{Volume of the emulsified fraction}}{\text{Total volume}} \times 100 \tag{3}$$

For ES evaluation, oil-in-water emulsions were prepared as described above. Fixed emulsion volumes of 40 mL were poured into centrifuge tubes and incubated at 80 °C for 30 min; they were subsequently centrifuged (Eppendorf™, Centrifuge 5810/5810R, Mexico) at 2500 rpm for 2 min, and the volume of the emulsified fraction was recorded. ES (%) was calculated according to Eq. (3).

2.2.4. Foaming capacity and foam stability

Foaming capacity (FC) and foam stability (FS) were determined using the method described by Coffman and García [19] with modifications. PbAm, AmPI, and SPI samples (0.5 g) were homogenized with 50 mL of distilled water. The solutions were adjusted to different pH values (1, 2, 3, 4, 5, 7, 9, 11, 12, 13) with 0.1 M HCl or 0.1 M NaOH. Subsequently, the solutions were homogenized with a high-speed turbo blender (Rhino, TURLIC-280, Mexico) at 20 000 rpm for 2 min. Foam volumes (mL) were recorded, and FC was calculated according to Eq. (4).

$$\text{FC}\ (\%) = \frac{\text{Foam volume}}{\text{Volume of solution before stirring}} \times 100 \tag{4}$$

FS was evaluated by measuring the remaining foam volume after the time interval (FV time t) at 5, 10, 15, 30 and 60 min at 25 °C, according to Eq. (5).

$$\text{FS}\ (\%) = \frac{\text{FV time t}}{\text{Volume of solution before stirring}} \times 100 \tag{5}$$

2.2.5. Oil absorption capacity

Oil absorption capacity (OAC) was determined using the method described by Lin et al. [20] with modifications. PbAm, AmPI, and SPI samples in proportions of 0.4, 0.9, 2.2, 3.5, 4.0, 6.0, 8.0, and 10.0 % (w/v) were mixed with 5 mL of neat canola oil. The solution was vortexed (IKA®, VORTEX 2, USA) for 10 min and centrifuged at 4000 rpm for 15 min, the supernatant was decanted, and the residue was weighed. OAC was calculated according to Eq. (6) and was expressed as a percentage.

$$\text{OAC}\ (\%) = \frac{\text{Weight of oil retained}}{\text{Initial weight of sample}} \times 100 \tag{6}$$

2.3. Obtaining the meat sausage

Three batches of meat sausage were prepared for each treatment by partially substituting SPI, based on the following treatments: T1 (15 % AmPI, 85 % SPI), T2 (10 % AmPI, 90 % SPI), T3 (5 % AmPI, 95 % SPI), and T4 (0 % AmPI, 100 % SPI) as the control. The meat paste preparation process was carried out in a food processor (Ninja Intelli®, BN801, Mexico), initially incorporating the meat (previously ground with a 3/8″ sieve), seasonings, and nitrites (dissolved in 70 g of cold water). After 1 min the fat, phosphates (dissolved in 30 g of cold water), 50 % of the retainers (AmPI and SPI), and ice were added, processed for 1 min, and then the remaining 50 % was incorporated. The process continued until reaching a total time of 5 min. The meat pastes obtained were stuffed into collagen casings (26 mm caliber) using a manual sausage stuffer (Estilo Modern, 无 brand). The sausage was cooked in a bain-marie at 80 °C until it reached an internal temperature of 71 °C, and then maintained at that temperature for 15 min. Subsequently, the meat sausage was cooled in a cold-water bath for 20 min, packaged, and stored at 4 °C. All analyses were conducted after 24 h of refrigeration.

2.3.1. Emulsion stability of meat pastes

The emulsion stability of meat pastes (ES-Mp) was determined according to the method of Hughes et al. [21], estimating the percentage of total fluid released (% TFR; using Eqs. (7) and (8)), percentage of fat released (% FR; Eq. (9)), and percentage of water released (% WR; Eq. (10)):

$$\text{TFR} = (\text{Weight of tube} + \text{sample}) - (\text{Weight of tube} + \text{sediment}) \tag{7}$$

$$\text{TFR}\ (\%) = \frac{\text{TFR}}{\text{Sample weight}} \times 100 \tag{8}$$

$$\text{FR}\ (\%) = \frac{(\text{Weight of crucible} + \text{dried supernatant}) - (\text{Weight of empty crucible})}{\text{TFR}} \times 100 \tag{9}$$

$$\text{WR}\ (\%) = \frac{(\text{Weight of crucible} + \text{sample}) - (\text{Weight of crucible} + \text{dried supernatant})}{\text{TFR}} \times 100 \tag{10}$$

2.3.2. Water absorption capacity of meat pastes

The water absorption capacity of meat pastes (WAC-Mp) was determined using the method described by Lin and Huang [22] with slight modifications. Meat paste samples (5 g) were homogenized with 10 mL of distilled water in a vortex (IKA®, VORTEX 2, USA) at 1200 rpm for 1 min. They were then centrifuged (Eppendorf™, Centrifuge 5810/5810R, Mexico) for 15 min at 4000 rpm, the supernatant was decanted, and the final residue was weighed. WAC-Mp was calculated according to Eq. (11) and was expressed in g of water absorbed per g of meat.

$$\text{WAC} - \text{Mp}\left(\frac{\text{g } H_2O \text{ absorbed}}{\text{g meat}}\right) = \frac{(\text{Final weight of sample} \ / \ \text{Initial weight of sample})}{\text{Initial weight of sample}} \times 100 \tag{11}$$

2.3.3. Physicochemical characterization of meat sausage

Cooking loss and yield were determined using the methodology described by Choi et al. [23]. The meat sausage was weighed before cooking, after cooking, and after storage at 4 °C for 24 h. Cooking loss and yield were calculated according to Eqs. (12) and (13), respectively.

$$\text{Cooking loss } (\%) = \frac{(\text{Weight before cooking} - \text{Weight after cooking})}{\text{Weight before cooking}} \times 100 \tag{12}$$

$$\text{Cooking yield } (\%) = \frac{\text{Weight after storage}}{\text{Weight before cooking}} \times 100 \tag{13}$$

The color evaluation of meat sausages was performed after 10 min of blooming time at room temperature (25 °C) using a Minolta colorimeter with the D65 illuminant, 10 standard observer, and an 8 mm of aperture in the observer (Konica Minolta Sensing, Inc., CR-400, Osaka, Japan). Results were expressed according to the color parameters L*, a*, and b*. The total color difference (ΔE) of the treatments (T4-T2) was calculated with respect to T1 using equation (14).

$$\Delta E = \sqrt{(L_0\text{-}L_1)^2 + (a_0\text{-}a_1)^2 + (b_0\text{-}b_1)^2} \tag{14}$$

Where the subscript 0 refers to the color reading of samples (T4-T2), while subscript 1 refers to the color of samples from T1.

Water activity (a_w) was determined at 25 °C using an a_w meter (Aqualab Pawkit, Meter Group Inc., USA). For pH measurement, 5 g of ground meat sausage was homogenized with 45 mL of distilled water. An Edge® multiparametric pH meter (HANNA® instruments, Costa Rica) calibrated with pH 4 and pH 7 buffer solutions at 25 °C was used. The electrode was inserted into the homogenized mixture, and the reading was recorded once the electrode had stabilized.

2.3.4. Proximal characterization of meat sausage

The proximal composition was determined according to the official methods of the Association of Official Analytical Chemists [13]. Moisture content was calculated by weight loss through oven drying at 103 ± 2 °C for 24 h (Method 950.46); fat content was determined by Soxhlet extraction using petroleum ether as a solvent (Method 31.4.02); protein content was determined by the Kjeldahl method (Method 992.15) using a conversion factor of 6.25; and ash content was determined in a muffle furnace at 600 °C (Method 923.03). Carbohydrates content was calculated by difference according to Eq. (15).

$$\text{Carbohydrates } (\%) = 100 - (\text{Moisture} + \text{Protein} + \text{Fat} + \text{Ash } \%) \tag{15}$$

2.3.5. Texture profile analysis and Warner-Bratzler shear force

Meat sausages from the different treatments were evaluated after 24 h of refrigeration (4 °C) and kept at room temperature (25 °C) for 1 h prior to measurement. Texture profile analysis (TPA) was performed using a texturometer (Shimadzu EZ-SX 500 N, Japan) with compression plates (118 mm diameter) connected to the load cell with a force of 500 N. Cylindrical slices (20 × 20 mm) of the meat sausages were subjected to a two-cycle compression up to 50 % of their original height, with a speed of 2 mm/s and a waiting time of 1 s between each compression, using a force of 500 N. Deformation curves over time and values for the texture profile parameters, including hardness (N), elasticity (mm), chewiness (N.mm), gumminess (N), and cohesion, were obtained using TRAPEZIUM2 version 2.20 software (Copyright 1999–2003). For Warner-Bratzler shear force (WBSF) analysis, meat sausages were cut into central segments of 30 mm long and 20 mm in diameter. Then, a Warner-Bratzler knife connected to the load cell was used with a force of 500 N, at a speed of 2 mm/s and a displacement of 30 mm. The maximum WBSF was considered as the maximum point recorded in the graph and expressed in Newtons (N).

2.3.6. Amino acid profile of meat sausage treatments T1 and T4

The amino acid profile of meat sausage treatments T1 and T4 was determined by HPLC using the INS-SM/US-260 method for total amino acids in foods, flours, and protein concentrates.

2.4. Statistical analysis

The evaluation of the functional properties of PbAm, AmPI, and SPI was carried out in three experiments using completely randomized designs (CRD) and general linear models (GLM). All analyzes were performed in triplicate ($n = 3$).

In the first experiment, PS, WAC, EC, and FC were evaluated, based on two fixed effects: experimental units (PbAm, AmPI, SPI) and pH (1, 2, 3, 4, 5, 7, 9, 11, 12, 13), as well as the interaction between both fixed effects, and the replicate (batch) as a random term. In the second experiment, only FS was evaluated. Three fixed effects were considered: experimental units (PbAm, AmPI, SPI), pH (3, 7, 12) and time (0, 5, 10, 15, 30 and 60 min), as described in section (2.2.4). The interaction between the fixed effects and the replicate (batch) as a random term was also analyzed. Finally, in the third experiment, the OAC was evaluated, based on two fixed effects: experimental units (PbAm, AmPI, SPI) and concentration percentages (%). These percentages were: 0.4, 0.9, 2.2, 3.5, 4.0, 6.0, 8.0 and 10.0 % (w/v), as described in section (2.2.5). Likewise, the interaction between the fixed effects and the replicate (batch) as a random term was analyzed.

The meat pastes and meat sausages described in section (2.3) were obtained by partially replacing SPI with AmPI. They were

prepared based on four treatments (T1, T2, T3 and T4) each performed in triplicate ($n = 3$) and analyzed after 24 h in refrigerated at 4 °C. A CRD was used to evaluate the properties of the meat pastes. The response variables analyzed were: TFR, FR, WR and WAC-Mp. For the analysis, a GLM was applied, considering the treatment as a fixed effect and the replicate (batch) as a random term. For the evaluation of meat sausages, a CRD was used. The response variables analyzed included physicochemical and proximal characterization, TPA, and WBSF. For the analysis, a GLM was applied, considering the treatment as a fixed effect and the replicate (batch) as a random term.

Finally, to evaluate the amino acid profile of the meat sausages, the T1 (15 % AmPI, 85 % SPI) and T4 (0 % AmPI, 100 % SPI) treatments were selected. T1 presented the highest inclusion of AmPI, while T4 was used as control (100 % SPI). The amino acid profile was analyzed using the same design and model (CRD and GLM, respectively) mentioned above, considering the treatment as a fixed effect and the replicate (batch) as a random term.

For each of the experiments, the normality of the data was verified with a Shapiro-Wilk test, which allows analyzing samples in a range of $3 \leq n \leq 5000$ [24]. To verify the homogeneity of the variance of the data, the Levene statistical test was performed; allows comparison of homoscedasticity between groups to determine the precision of an Analysis of variance (ANOVA) [25]. ANOVA was performed for each of the response variables evaluated. The comparison of means was performed using Tukey's test for a significance level of 5 % ($p < 0.05$). Data analysis was performed using statistical software Minitab Inc., State College, PA, USA.

3. Results and discussion

3.1. Obtaining powder biomass and protein isolate from Arthrospira maxima

Powdered biomass of *A. maxima* (PbAm) yield (10.13 %) is associated with the conversion rate from fresh biomass to dry biomass, due to the nature of *A. maxima*, which has a high water content. Therefore, after drying, PbAm presented a low moisture content (10.72 %), a value that is within the range reported by Official Mexican Standard NMX-F-508-1988 [26] for the conservation and stability of *Spirulina*. The alkaline solubilization and isoelectric precipitation method used allowed the isolation of an *A. maxima* protein isolate (AmPI) with a yield of 33.37 % (w/w). Similar results were reported by Pereira et al. [1] in a *Spirulina* protein isolate (33 %) obtained by chemical extraction.

3.1.1. Proximal composition

The proximal composition of PbAm and AmPI is presented in Table 1. Results obtained were consistent with those reported by Lupatini et al. [8] in *A. platensis* biomass (59.50 % protein, 0.81 % fat, 13.25 % ash, and 26.44 % carbohydrates). However, the proximal composition of microalgae can vary depending on the genus, species, culture, or environmental conditions [27].

The moisture, fat, ash, and carbohydrate contents of AmPI were lower with respect to PbAm, while the protein content of AmPI (82.58 %) showed an increase of 21.62 % compared to PbAm (60.96 %). The protein content of AmPI is higher than that reported by Benelhadj et al. [28] for an *A. platensis* protein isolate (69.62 %). The protein extraction yield is influenced by the efficiency of pretreatments and cell disruption techniques, as well as the characteristics of microalgae cell walls and the nature of their proteins, both cytosolic and structural [29]. These factors determine their ability to be released or remain trapped in the phase of cellular remnants [8].

3.1.2. Structural characterization

The FTIR spectra of PbAm, AmPI, and soy protein isolate (SPI) in the 700 to 2000 cm^{-1} region are presented in Fig. 1(a–c). Spectral regions associated with the presence of proteins, carbohydrates, and lipids were identified, with some similarities among the absorption bands.

In general, characteristic bands of amide I at 1647 cm^{-1} and amide II at 1539 cm^{-1} were observed, induced by stretching vibrations of the C═O and N–H bonds, reflecting the secondary structure of the proteins. In the region of 800–1200 cm^{-1}, the presence of carbohydrates associated with C–O–C and C–O bonds was detected, while bands at 1457 and 1747 cm^{-1} confirmed the presence of lipids corresponding to CH_3 and C═O bonds.

The spectra of AmPI and SPI exhibited higher absorbance bands in the regions of amide I and amide II. In contrast, a decrease in the intensity of bands related to carbohydrates was observed in the region of 800–1200 cm^{-1}, suggesting a higher concentration of these in AmPI compared to SPI. These results indicate that, although AmPI and SPI have similar spectra, they differ in protein and carbohydrate

Table 1
Proximal composition of powdered biomass of *Arthrospira maxima* (PbAm) and *Arthrospira maxima* protein isolate (AmPI).

Proximate parameters (%)	PbAm	AmPI
Yield	10.13 ± 0.32	33.37 ± 1.33
Moisture	10.72 ± 0.15	8.73 ± 0.02
Protein	60.96 ± 0.51	82.58 ± 0.51
Fat	1.28 ± 0.04	0.71 ± 0.01
Ash	9.64 ± 0.14	3.35 ± 0.17
Carbohydrates	17.4 ± 0.57	4.63 ± 0.38

Each value represents the mean ± standard deviation, $n = 3$.

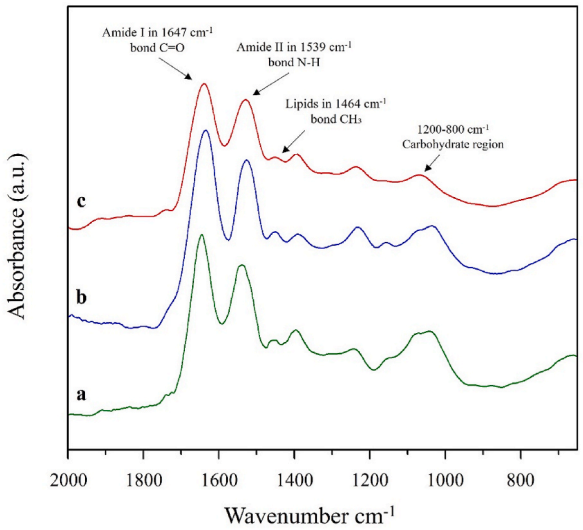

Fig. 1. FTIR spectra; **a.** powdered biomass of *Arthrospira maxima* (PbAm), **b.** *Arthrospira maxima* protein isolate (AmPI), and **c.** soy protein isolate (SPI) in the 700-2000 cm^{-1} region.

content. Similar findings were reported by Lupatini et al. [8] in a protein concentrate of *A. platensis*.

3.1.3. Amino acid profile

The proteins of microalgae of the genus *Arthrospira* present a balanced distribution of essential and non-essential amino acids and are classified as complete proteins by containing all the essential amino acids [30]. The amino acid profile of AmPI was compared with PbAm and SPI [31], the results are presented in Table 2.

The essential amino acids of PbAm and AmPI recorded high contents of leucine, followed by valine and isoleucine, while methionine and tryptophan showed the lowest contents. The contribution of essential amino acids was comparatively higher in AmPI (23.97

Table 2
Amino acid profile of powdered biomass of *Arthrospira maxima* (PbAm) compared to *Arthrospira maxima* protein isolate (AmPI) and soy protein isolate (SPI).

Amino acids (g/100 g)	PbAm	AmPI	SPI[a]
Essential			
Valine	3.29 ± 0.00	3.57 ± 0.00	4.20 ± 0.13
Threonine	2.87 ± 0.00	3.07 ± 0.00	3.62 ± 0.15
Isoleucine	3.18 ± 0.00	3.69 ± 0.00	4.29 ± 0.08
Leucine	6.34 ± 0.00	7.38 ± 0.00	7.99 ± 0.27
Lysine	2.09 ± 0.00	2.31 ± 0.00	5.26 ± 0.17
Methionine	0.001 ± 0.00	0.001 ± 0.00	0.75 ± 0.12
Histidine	0.81 ± 0.00	0.92 ± 0.00	2.49 ± 0.05
Phenylalanine	2.61 ± 0.00	3.03 ± 0.00	5.32 ± 0.38
Tryptophan	0.01 ± 0.00	0.002 ± 0.00	–
Non-essential			
Glycine	2.47 ± 0.00	2.86 ± 0.00	3.80 ± 0.12
Alanine	6.51 ± 0.00	6.87 ± 0.00	4.01 ± 0.14
Serine	3.12 ± 0.00	3.35 ± 0.00	4.95 ± 0.22
Proline	4.82 ± 0.00	6.01 ± 0.00	4.86 ± 0.37
Cysteine	0.01 ± 0.00	0.001 ± 0.00	0.15 ± 0.07
Asparagine	0.001 ± 0.00	0.001 ± 0.00	–
Aspartic acid	5.66 ± 0.00	6.37 ± 0.00	4.95 ± 0.22
Glutamine	0.001 ± 0.00	0.001 ± 0.00	–
Glutamic acid	8.01 ± 0.00	8.12 ± 0.00	17.85 ± 0.67
Arginine	5.47 ± 0.00	5.82 ± 0.00	7.36 ± 0.15
Tyrosine	2.59 ± 0.00	3.02 ± 0.00	3.49 ± 0.10
Totals	59.85 ± 0.004	66.39 ± 0.001	85.34 ± 3.84
Essential	21.19 ± 0.001	23.97 ± 0.00	33.92 ± 2.14
Non-essential	38.66 ± 0.003	42.42 ± 0.001	51.42 ± 4.93
E/NE ratio	0.55 ± 0.00	0.57 ± 0.00	0.66 ± 0.00

Each value represents the mean ± standard deviation, $n = 3$.
[a] Zhang et al. [31].

g/100 g) than in PbAm (21.19 g/100 g). Regarding non-essential amino acids, glutamic acid followed by alanine, aspartic acid, and arginine exhibited the highest values, while cysteine, asparagine, and glutamine presented the lowest values. A comparison of non-essential amino acid profiles showed higher contents for AmPI (42.42 g/100 g) than for PbAm (38.66 g/100 g). Similar results were reported by Bashir et al. [30] and Lupatini et al. [8], who determined that *S. platensis* protein concentrates have higher amino acid contents than the powdered biomass. The amino acid profile of AmPI compared to SPI showed lower values, except for alanine, proline, and aspartic acid. Because AmPI contains all the essential amino acids, it can be considered a source to provide these nutrients.

3.2. Functional properties

The effect of pH on the functional properties of protein solubility (PS), water absorption capacity (WAC), emulsifying capacity (EC), emulsifying stability (ES), and foaming capacity (FC) of PbAm, AmPI, and SPI is shown in Table 3.

The minimum PS was detected at pH 3 for PbAm and AmPI, while for SPI it was at pH 4, which corresponds to the isoelectric point (pI) of the evaluated proteins. In contrast, the maximum PS reached was observed at pH 12. AmPI compared to PbAm showed a significant increase ($p < 0.05$), reaching values comparable to those of SPI. In general, PS increased significantly ($p < 0.05$) at pH values above and below the respective pI for each sample. This increase is attributed to the electrostatic repulsions resulting from changes in the charges of the side chains of amino acids, as well as the ionic hydration forces between the protein molecules. Lower PS values in a protein isolate of *A. platensis* obtained by chemical extraction were reported by Benelhadj et al. [28].

The highest WAC for SPI was recorded at pH 11, while for PbAm and AmPI it was at pH 12, with statistically significant differences ($p < 0.05$). The WAC of AmPI was more than double that of PbAm. This increase could be attributed to numerous surface hydrophilic binding sites, as pH changes lead to the exposure or capture of water-binding sites [32]. The minimum WAC was obtained at pH 3 for PbAm and AmPI, while for SPI it was at pH 13; pH changes close to the pI or that are extremely alkaline affect the protein structure, causing the burial of hydrophilic binding sites [27].

The maximum EC was recorded at pH 7 for PbAm and AmPI, and at pH 9 for SPI, with statistically significant differences ($p < 0.05$). At pH 4–5 for SPI and pH 3–4 for PbAm and AmPI, the emulsions were not formed because at these pH values the proteins present their minimum solubility, preventing them from rapidly moving to the water/oil interface. The electrical charge of the proteins decreased, causing a low intensity of the electrostatic repulsive forces between the oil droplets that were associated to form aggregates [28,33].

As for the ES, the emulsions formed as a function of pH (1–13) were not significantly affected ($p > 0.05$) by the heat treatment (80 °C) to which they were subjected for 30 min.

At pH above and below the isoelectric pH, the emulsions were stable to cream formation and flocculation. However, at isoelectric

Table 3
Functional properties of powdered biomass of *Arthrospira maxima* (PbAm), *Arthrospira maxima* protein isolate (AmPI) and soy protein isolate (SPI) as a function of pH.

Functional property (%)		pH									
		1	2	3	4	5	7	9	11	12	13
Protein solubility (PS)	PbAm	21.88 ± 0.03^{q}	17.81 ± 0.66^{r}	3.20 ± 0.65^{t}	24.99 ± 0.54^{op}	31.28 ± 0.30^{m}	42.83 ± 1.48^{j}	49.83 ± 0.55^{h}	58.39 ± 0.25^{g}	62.25 ± 0.19^{f}	48.82 ± 0.63^{hi}
	AmPI	30.65 ± 0.13^{mn}	22.92 ± 0.14^{pq}	9.13 ± 0.32^{s}	34.60 ± 0.09^{l}	44.45 ± 0.22^{j}	56.64 ± 0.14^{g}	68.50 ± 0.46^{e}	82.87 ± 0.04^{b}	87.78 ± 0.31^{a}	73.81 ± 0.37^{d}
	SPI	50.47 ± 0.60^{h}	45.8 ± 0.89^{ij}	27.95 ± 0.28^{no}	11.60 ± 0.09^{s}	39.06 ± 0.81^{k}	51.01 ± 1.42^{h}	64.65 ± 0.40^{f}	79.60 ± 0.22^{c}	86.05 ± 0.45^{a}	79.72 ± 0.21^{c}
Water absorption capacity (WAC)	PbAm	224.25 ± 0.63^{t}	284.55 ± 0.45^{p}	167.47 ± 0.57^{v}	184.28 ± 0.78^{w}	218.30 ± 0.67^{u}	306.25 ± 0.46^{o}	339.76 ± 0.69^{m}	361.48 ± 0.71^{k}	400.26 ± 0.82^{j}	334.64 ± 0.43^{n}
	AmPI	273.95 ± 0.49^{q}	363.92 ± 0.46^{k}	219.47 ± 0.73^{u}	260.59 ± 0.64^{r}	276.73 ± 0.40^{q}	357.82 ± 0.69^{l}	513.69 ± 0.65^{i}	679.14 ± 0.44^{g}	828.49 ± 0.48^{e}	636.07 ± 0.29^{h}
	SPI	744.15 ± 0.24^{f}	983.96 ± 0.69^{c}	639.20 ± 0.39^{h}	228.58 ± 0.79^{s}	306.84 ± 0.22^{o}	916.69 ± 0.63^{d}	1121.32 ± 0.94^{b}	1428.75 ± 0.61^{a}	43.71 ± 0.34^{x}	16.88 ± 0.57^{y}
Emulsifying capacity (EC) and stability (ES)	PbAm	48.25 ± 0.29^{i}	50.17 ± 0.30^{h}	0.00 ± 0.00^{q}	0.00 ± 0.00^{q}	50.25 ± 0.29^{h}	59.92 ± 0.36^{c}	56.42 ± 0.22^{d}	47.67 ± 0.30^{ij}	39.33 ± 0.30^{n}	28.04 ± 0.11^{p}
	AmPI	43.75 ± 0.14^{l}	47.96 ± 0.11^{ij}	0.00 ± 0.00^{q}	0.00 ± 0.00^{q}	55.08 ± 0.22^{ef}	69.79 ± 0.15^{a}	66.88 ± 0.31^{b}	55.17 ± 0.36^{def}	46.33 ± 0.22^{k}	34.08 ± 0.15^{o}
	SPI	46.79 ± 0.29^{jk}	54.58 ± 0.22^{f}	47.92 ± 0.22^{ij}	0.00 ± 0.00^{q}	0.00 ± 0.00^{q}	56.17 ± 0.22^{de}	67.08 ± 0.25^{b}	59.75 ± 0.14^{c}	52.75 ± 0.52^{g}	42.21 ± 0.23^{m}
Foaming capacity (FC)	PbAm	132.20 ± 1.96^{opq}	122.73 ± 1.57^{qr}	104.98 ± 1.89^{s}	115.69 ± 1.70^{r}	130.00 ± 1.73^{opq}	138.89 ± 1.60^{mno}	148.70 ± 1.58^{klm}	166.67 ± 1.28^{hi}	176.06 ± 1.63^{gh}	152.32 ± 1.28^{jkl}
	AmPI	165.48 ± 2.84^{i}	146.47 ± 1.95^{lmn}	126.40 ± 1.44^{pq}	138.89 ± 1.18^{mno}	158.97 ± 0.32^{ijk}	179.83 ± 2.11^{fg}	192.50 ± 1.44^{de}	226.13 ± 2.08^{b}	238.10 ± 1.25^{a}	214.64 ± 0.98^{c}
	SPI	145.74 ± 1.48^{lmn}	149.40 ± 2.59^{kl}	135.19 ± 2.14^{op}	122.96 ± 2.46^{qr}	137.91 ± 2.29^{no}	147.67 ± 2.33^{lmn}	160.52 ± 2.53^{ij}	187.88 ± 2.64^{def}	194.89 ± 1.64^{d}	183.86 ± 1.61^{efg}

Each value represents the mean ± standard error, $n = 3$. ^{abcd}, Different superscripts in the same rows of each functional property indicate statistically significant differences ($p < 0.05$).

pH, the emulsions failed to form, and the complete separation of the phases was observed when the samples were centrifuged. AmPI can be applied in the meat industry due to its ability to improve the formation and stability of meat emulsions characterized by having a pH of 5–7, where its EC was significantly higher ($p < 0.05$) than that of SPI (Table 3).

The maximum FC was recorded at pH 12, with statistically significant differences ($p < 0.05$) among the evaluated samples. The highest FC value of AmPI (238.10 %) was comparable to that of *S. platensis* protein concentrate (226.46 %, pH 8) [8]. The minimum FC was observed at pH 4 for SPI, while for PbAm and AmPI it was at pH 3. At values below or above the pI of the evaluated proteins, the FC increases, similar to the findings mentioned above for the functional properties of PS and WAC. This behavior is due to the increased electrical charge and the electrostatic repulsion caused by the net charges of the proteins that tend to diffuse rapidly at the air/water interface to surround the air bubbles [33]. Benelhadj et al. [28] reported that the FC of a *S. platensis* protein isolate is pH dependent, reaching values greater than 250 % at alkaline pH.

The effects of pH (3,7 and 12) on the foam stability (FS) of PbAm, AmPI, and SPI at different times (0–60 min) are shown in Fig. 2. The volume of foam recorded after 30 min of rest for the evaluated samples was higher ($p < 0.05$) at pH 12 compared to pH 3.

The FS of the evaluated samples decreased significantly ($p < 0.05$) over time at each tested pH level, with a drastic decrease occurring within the first 5 min, by approximately 100 %. The low FS could be attributed to the fact that PbAm, AmPI, and SPI failed to form dense and elastic interfacial films that would allow higher FS. The strong surface tension of the water caused the formed bubbles to break immediately; however, after 10 min, the foams remained stable for a longer time.

The oil absorption capacity (OAC) of PbAm, AmPI, and SPI was evaluated at concentrations ranging from 0.4 to 10.0 % (Fig. 3). The highest OAC values were recorded at a concentration of 0.4 % for PbAm, AmPI, and SPI (649.59 %, 736.15 %, and 814.79 %, respectively), with significant differences ($p < 0.05$). This was attributed to the higher availability of lipophilic groups, which facilitate oil penetration and retention in the evaluated samples. Meanwhile, the minimum OAC values were observed at a concentration of 10.0 %, with significant differences ($p < 0.05$). Lupatini et al. [8] reported similar results in a protein concentrate of *S. platensis* with a protein content of 75.97 %.

The differences in OAC among the samples can be mainly attributed to the variable presence of amino acids with non-polar side chains, which exhibit a greater affinity for lipid molecules [20]. Additionally, the protein extraction process contributes to higher OAC in the isolates compared to PbAm. During this process, the partial denaturation of proteins exposes their non-polar side chains, facilitating a more effective interaction with the aliphatic chains of lipids and consequently greater oil absorption [34].

3.3. Characterization of meat pastes and sausages

The emulsion stability of meat pastes (ES-Mp), expressed as total fluid released (TFR), fat released (FR), and water released (WR), is crucial for defining the quality of meat products. Table 4 shows significant differences ($p < 0.05$) among the evaluated treatments, where T4 exhibited the lowest values of TFR, FR, and WR, while T1 showed the highest. The increase in the inclusion of AmPI in the meat pastes could be associated with higher WR, possibly due to its lower water absorption capacity (WAC) (357.82 %) compared to SPI (916.69 %) at pH 7 (Table 3), which is close to the pH of meat products (pH 6.29), suggesting a similar behavior. Additionally, ES-

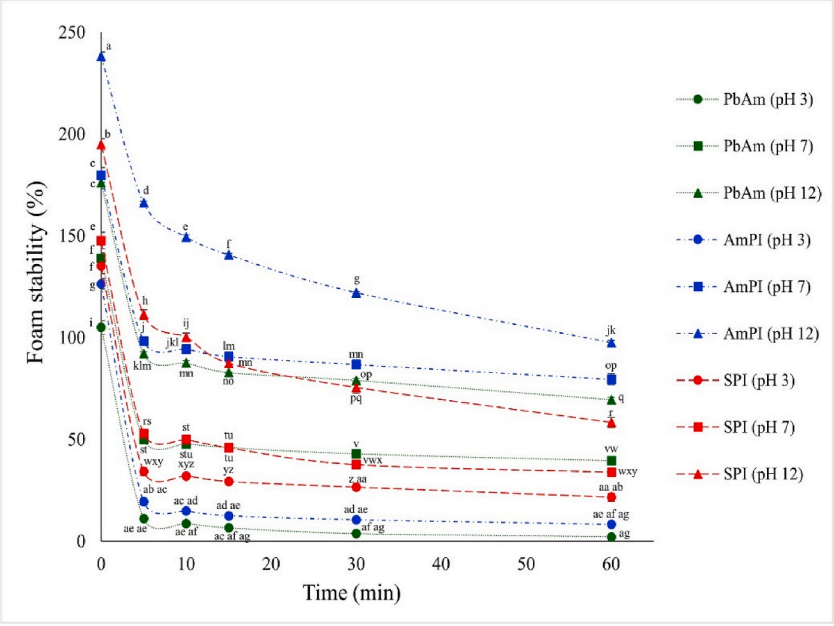

Fig. 2. Foam stability (FS) of powdered biomass of *Arthrospira maxima* (PbAm), *Arthrospira maxima* protein isolate (AmPI), and soy protein isolate (SPI). Each value represents the mean ± standard error, $n = 3$. ^{abcd}, Different superscripts indicate statistically significant differences ($p < 0.05$).

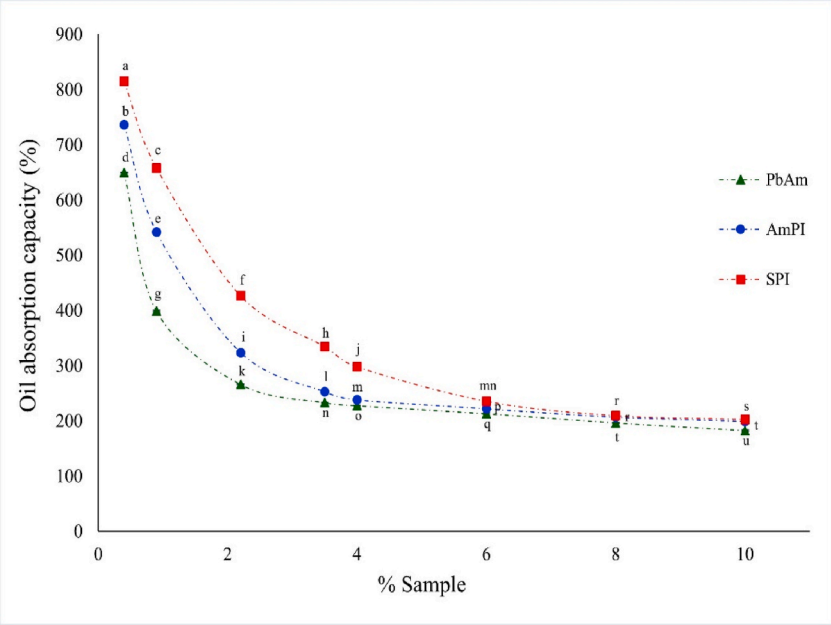

Fig. 3. Oil absorption capacity (OAC) of powdered biomass of *Arthrospira maxima* (PbAm), *Arthrospira maxima* protein isolate (AmPI), and soy protein isolate (SPI) in the range of 0.4–10.0 % of sample. Each value represents the mean ± standard error, $n = 3$. ^{abcd}, Different superscripts indicate statistically significant differences ($p < 0.05$).

Table 4
Emulsion stability of meat pastes (ES-Mp) formulated with the inclusion of AmPI at 15, 10 and 5 %, and control meat paste (100 % SPI).

Stability parameters	Treatments			
	T1	T2	T3	T4
Total fluid released (%)	6.95 ± 0.03^{a}	5.65 ± 0.02^{b}	4.22 ± 0.03^{c}	3.51 ± 0.03^{d}
Fat released (%)	0.66 ± 0.01^{a}	0.38 ± 0.01^{b}	0.23 ± 0.002^{c}	0.13 ± 0.001^{d}
Water released (%)	6.29 ± 0.03^{a}	5.27 ± 0.03^{b}	3.99 ± 0.03^{c}	3.37 ± 0.03^{d}
WAC-Mp (g H_2O absorbed/g meat)	0.20 ± 0.002^{a}	0.20 ± 0.001^{a}	0.20 ± 0.002^{a}	0.20 ± 0.001^{a}

T1: 15 % AmPI, 85 % SPI; T2: 10 % AmPI, 90 % SPI; T3: 5 % AmPI, 95 % SPI; T4: 0 % AmPI, 100 % SPI. AmPI: *Arthrospira maxima* protein isolate; SPI: soy protein isolate; WAC-Mp: Water absorption capacity of meat pastes. Each value represents the mean ± standard error, $n = 3$. ^{abcd}, different superscripts in the same row indicate statistically significant differences ($p < 0.05$).

Mp may be influenced by the lower protein content (82.58 %) and the presence of carbohydrates (4.63 %) in AmPI compared to SPI (94 % and <1 %, respectively). The interaction between proteins and high levels of carbohydrates in meat emulsions creates a thick gel network that retains water and oil. This could explain the lack of significant differences in WAC-Mp among the treatments (Table 4). However, during the cooking of the meat paste, the gel network compacts and strengthens, which could result in higher TFR [35]. This highlights the importance of ensuring an adequate balance between the purity, quality, and protein content of the isolates used [36].

Table 5 shows the physicochemical and proximal characterization, texture profile analysis (TPA) and Warner-Bratzler shear force (WBSF) of the meat sausages with partial inclusion of protein isolated from *A. maxima* (Fig. 4(a–d)). The cooking losses and cooking yield of meat sausages are mainly related to the parameters of ES-Mp formulated during heat treatment. Although the values obtained did not show statistically significant differences ($p > 0.05$), it was observed that with the increase in concentration of AmPI the cooking losses decreased and consequently the cooking yield increased.

The color parameters (L* and a*) of meat sausages were significantly affected ($p < 0.05$) by the inclusion of AmPI (Table 5). T1 had the lowest L* and a* color values compared to T4, which had a lighter and yellower color. Additionally, our findings align with those of Parniakov et al. [37], who reported that the total substitution of soy protein with microalgae protein (*Spirulina* and *Chlorella*) in meat sausages resulted in lower values of L* and a* due to the presence of photosynthetic pigments such as chlorophyll, phycocyanin, and carotenoids.

However, it's noteworthy that the b* index (yellowness levels) was not significantly affected ($p > 0.05$) in T1, T2, and T3 compared to T4, due to the absence of AmPI.

Fig. 4(a-c) illustrates how the inclusion of AmPI acts as a coloring agent in meat sausages, confirmed by the total color difference (ΔE) of the treatments (T4 = 20.79, T3 = 13.08, and T2 = 11.68) with respect to T1, values that exceeded the human perception threshold established at $\Delta E > 3$ [38]. Bošković et al. [12] reported a ΔE of 17.47 when adding 3 % honey *Chlorella vulgaris* to a meat

Table 5
Physicochemical and proximal characterization, texture profile analysis (TPA) and Warner-Bratzler shear force (WBSF) of meat sausages with the inclusion of AmPI at 15, 10 and 5 %, and control meat sausage (100 % SPI).

		Treatments			
		T1	T2	T3	T4
Physicochemical parameters					
Cooking loss (%)		2.79 ± 0.11^{a}	2.95 ± 0.11^{a}	3.08 ± 0.10^{a}	3.25 ± 0.20^{a}
Cooking yield (%)		97.29 ± 0.11^{a}	97.14 ± 0.11^{a}	97.02 ± 0.09^{a}	96.86 ± 0.19^{a}
Color	L*	59.28 ± 0.5^{d}	67.38 ± 0.4^{c}	69.80 ± 0.4^{b}	78.22 ± 0.3^{a}
	a*	-7.48 ± 0.2^{d}	-6.20 ± 0.2^{c}	-4.80 ± 0.1^{b}	1.00 ± 0.0^{a}
	b*	18.08 ± 0.1^{a}	18.48 ± 0.5^{a}	18.21 ± 0.2^{a}	18.78 ± 0.1^{a}
	ΔE	20.79 ± 1.34	13.08 ± 0.59	11.68 ± 0.65	–
a_w		0.99 ± 0.002^{a}	0.99 ± 0.002^{a}	0.99 ± 0.001^{a}	0.99 ± 0.001^{a}
pH		6.28 ± 0.002^{a}	6.29 ± 0.002^{a}	6.29 ± 0.002^{a}	6.28 ± 0.002^{a}
Proximal parameters					
Moisture (%)		65.73 ± 0.63^{a}	66.18 ± 0.26^{a}	66.45 ± 0.14^{a}	66.43 ± 0.18^{a}
Fat (%)		13.49 ± 0.24^{a}	13.17 ± 0.23^{a}	13.18 ± 0.18^{a}	13.04 ± 0.16^{a}
Protein (%)		12.55 ± 0.22^{d}	13.79 ± 0.06^{c}	14.81 ± 0.06^{b}	16.33 ± 0.02^{a}
Ash (%)		1.89 ± 0.02^{b}	1.93 ± 0.01^{b}	1.93 ± 0.004^{b}	1.99 ± 0.01^{a}
Carbohydrates (%)		6.34 ± 0.17^{a}	4.93 ± 0.07^{b}	3.63 ± 0.03^{c}	2.22 ± 0.06^{d}
TPA parameters					
Hardness (N)		21.24 ± 0.38^{a}	22.544 ± 0.355^{a}	21.41 ± 0.29^{a}	21.38 ± 0.35^{a}
Elasticity (mm)		1.04 ± 0.03^{a}	1.034 ± 0.022^{a}	1.02 ± 0.01^{a}	1.04 ± 0.02^{a}
Chewiness (N)		8.39 ± 0.24^{a}	8.415 ± 0.284^{a}	7.96 ± 0.12^{a}	8.69 ± 0.19^{a}
Gumminess (N)		8.12 ± 0.25^{a}	8.187 ± 0.208^{a}	7.79 ± 0.12^{a}	8.44 ± 0.41^{a}
Cohesion (dimensionless)		0.25 ± 0.03^{a}	0.352 ± 0.080^{a}	0.32 ± 0.02^{a}	0.23 ± 0.06^{a}
WBSF parameter					
Shear force (N)		5.23 ± 0.10^{a}	5.462 ± 0.062^{a}	5.53 ± 0.02^{a}	5.28 ± 0.10^{a}

T1: 15 % AmPI, 85 % SPI; T2: 10 % AmPI, 90 % SPI; T3: 5 % AmPI, 95 % SPI; T4: 0 % AmPI, 100 % SPI. AmPI: *Arthrospira maxima* protein isolate; SPI: soy protein isolate. ΔE: color differences of the treatments (T4-T2) with respect to T1. Each value represents the mean ± standard error, $n = 3$. ^{abcd}, different superscripts in the same row indicate statistically significant difference ($p < 0.05$).

Fig. 4. Visual appearance of formulated meat sausages **a.** T1 (15 % AmPI, 85 % SPI), **b.** T2 (10 % AmPI, 90 % SPI), **c.** T3 (5 % AmPI, 95 % SPI), **d.** T4 (0 % AmPI, 100 % SPI). AmPI: *Arthrospira maxima* protein isolate; SPI: soy protein isolate.

sausage. It has been demonstrated that the inclusion of microalgae in meat products affects their sensory characteristics and, consequently, the acceptance of the developed products [12,37,39]. This influence could be attributed to the limited familiarity of the panelists with the products. However, according to Cofrades et al. [40], in countries where the consumption of algae and microalgae is common, their characteristic taste and color would not be an obstacle to the acceptance of meat products but could improve it. Furthermore, an alternative involves extracting the photosynthetic pigments using solvents such as ethanol, acetone, and water to enhance the sensory properties of foods containing microalgae proteins [41].

The pH, a_w, moisture, and fat did not show statistically significant differences ($p > 0.05$) among the treatments. The protein content was significantly higher ($p < 0.05$) in T4 and the lowest in T1. However, none of the treatments presented contents lower than the minimum amount indicated in the Official Mexican Standard NMX-F-065-1984 [42], which establishes 9.5 % protein in meat sausages. These results are consistent with those reported by Parniakov et al. [37] and Marti-Quijal et al. [5] for the proximal parameters. They also observed that meat products with soy protein have significantly higher protein content ($p < 0.05$) compared to those containing microalgae proteins such as *Spirulina* and *Chlorella*.

The ash content in T4 was significantly higher ($p < 0.05$) than in the other treatments, while the carbohydrate content increased as the concentration of AmPI in the meat sausages increased ($p < 0.05$). Bošković et al. [12] reported similar ash contents (1.96–2.04 %) in pork sausages enriched with white and honey *C. vulgaris*, showing an increase in carbohydrates, although less than in our study. The inclusion of microalgae increases the carbohydrate content in meat sausages due to the accumulation of starch inside the cell as the main storage component [43].

The TPA and WBSF values are shown in Table 5. All treatments exhibited similar values ($p > 0.05$), indicating that the partial inclusion of AmPI had no effect on the textural properties analyzed compared to T4. This behavior is possibly due to the functional properties presented by AmPI, whose EC evaluated at pH 6–7 (Table 3) was significantly higher ($p < 0.05$) compared to SPI. Therefore, AmPI at pH 6.29 in T1, T2, and T3 provided a high ES that allowed the protein matrix and continuous phase of meat emulsions to be maintained, as in T4 [38]. Similar results were reported by Marti-Quijal et al. [5] in fresh pork sausages made with *Spirulina* protein.

Table 6 shows the amino acid content of meat sausage T1 and T4. The content of cysteine, methionine, asparagine, and tryptophan showed no significant difference ($p > 0.05$) between treatments; however, they presented the lowest concentrations (<0.002 g/100 g). Non-essential amino acids such as glutamic acid and proline showed the highest concentrations in both treatments. Glutamic acid was significantly higher ($p < 0.05$) in T4, while proline presented higher values in T1.

The essential amino acids lysine and leucine were in both treatments. Lysine presented significantly higher values ($p < 0.05$) in T1, while leucine was significantly higher ($p < 0.05$) in T4. The highest values of total, essential, and non-essential amino acids were presented in T1, while T4 showed lower values ($p < 0.05$). The ratio of essential to non-essential amino acids was higher in T4 than in T1. Thus, the inclusion of AmPI in the formulation of meat sausages resulted in a higher amino acid content compared to SPI.

Several authors have reported that adding microalgae (*Spirulina* and *Chlorella*) at low concentrations to meat products increases the content of most amino acids [5,37,44]. Therefore, this enrichment could generate important health benefits, mainly by satisfying the body's metabolic demands for essential amino acids [40].

4. Conclusion

A. maxima protein isolate (AmPI) was obtained with a protein content of 82.58 %, evidencing its great potential as an alternative protein source, due to its amino acid profile. AmPI outperforms powdered biomass of *A. maxima* (PbAm) and soy protein isolate (SPI) in terms of protein solubility (PS) and foaming capacity (FC) at pH > 5. Additionally, it demonstrates remarkable emulsifying capacity (EC) in the pH range of 5–7, rendering it suitable for enhancing techno-functional processes. However, functional properties near the isoelectric point (pI = 3) may present challenges in practical applications of AmPI under specific conditions. The partial inclusion of

Table 6
Amino acid composition (g/100 g) of meat sausages T1 (15 % AmPI, 85 % SPI) and T4 (0 % AmPI, 100 % SPI).

Amino acids (g/100 g)	Treatment	
	T1	T4
Essential		
Valine	4.16 ± 0.00[a]	3.99 ± 0.00[b]
Threonine	2.26 ± 0.00[a]	2.20 ± 0.00[b]
Isoleucine	3.85 ± 0.00[a]	3.81 ± 0.00[b]
Leucine	5.23 ± 0.00[b]	5.42 ± 0.00[a]
Lysine	5.86 ± 0.00[a]	5.75 ± 0.00[b]
Methionine	<0.001 ± 0.00	<0.001 ± 0.00
Histidine	2.94 ± 0.00[a]	2.70 ± 0.00[b]
Phenylalanine	4.01 ± 0.00[a]	3.97 ± 0.00[b]
Tryptophan	0.002 ± 0.00	0.002 ± 0.00
Non-essential		
Glycine	2.66 ± 0.00[a]	2.63 ± 0.00[b]
Alanine	4.72 ± 0.00[a]	3.97 ± 0.00[b]
Serine	2.64 ± 0.00[b]	2.68 ± 0.00[a]
Proline	8.04 ± 0.00[a]	6.99 ± 0.00[b]
Cysteine	<0.001 ± 0.00	<0.001 ± 0.00
Asparagine	<0.001 ± 0.00	<0.001 ± 0.00
Aspartic acid	5.04 ± 0.00[a]	4.70 ± 0.00[b]
Glutamine	0.02 ± 0.00[b]	0.03 ± 0.00[a]
Glutamic acid	8.49 ± 0.00[b]	8.60 ± 0.00[a]
Arginine	5.44 ± 0.00[a]	5.14 ± 0.00[b]
Tyrosine	2.96 ± 0.00[a]	2.75 ± 0.00[b]
Totals	68.32 ± 0.002[a]	65.35 ± 0.01[b]
Essential	28.31 ± 0.001[a]	27.85 ± 0.003[b]
Non-essential	40.01 ± 0.001[a]	37.50 ± 0.01[b]
E/NE ratio	0.71 ± 0.00[a]	0.74 ± 0.00[b]

AmPI: *Arthrospira maxima* protein isolate; SPI: soy protein isolate. Each value represents the mean ± standard error, $n = 3$. [abcd], Different superscripts in the same row indicate statistically significant differences ($p < 0.05$).

AmPI into the meat sausages did not affect their textural properties, although significant changes were observed in the L* and a* color parameters. The inclusion of 15 % AmPI shows considerable potential for improving the nutritional profile of meat sausages. Nonetheless, further research on the cellular disruption of *A. maxima* during protein extraction is required to optimize the rate, yield, and quality of the protein isolate. These advancements would not only contribute to the advancement of scientific knowledge but also unlock new opportunities in the food industry, particularly in terms of functional applications.

Data availability statement

Data associated with this study have not been deposited into a publicly available repository.
Data included in article/supp. material/referenced in article.

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CRediT authorship contribution statement

Mariana Inés Acateca-Hernández: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aleida S. Hernández-Cázares:** Writing – review & editing, Supervision, Resources, Conceptualization. **Juan Valente Hidalgo-Contreras:** Writing – review & editing, Validation, Resources. **María Teresa Jiménez-Munguía:** Writing – review & editing. **Ma. Antonieta Ríos-Corripio:** Writing – review & editing, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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