



# Cold-pressed sesame seed meal as a protein source: Effect of processing on the protein digestibility, amino acid profile, and functional properties

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## ABSTRACT

Alternative protein sources for the human diet may help overcome the food security challenge for a growing population and its environmental impact. Edible oil extraction by-products are potential sources due to high protein content. This study characterized cold-pressed sesame seed meals about the proximate composition, antinutritional factors, amino acid profile and score, and in vitro protein digestibility (IVPD). A central composite experimental design supported evaluating the impact of cooking, microwave, and ultrasound on the nutritional quality of this meal. The raw sample presented 35% of protein and 89% of IVPD. Optimized conditions were 87.8 °C, pH 8.0, and 37 min for all processes studied, increasing IVPD up to 95%. The treatments effectively reduced up to 55% trypsin inhibitor activity and 81% phytic acid content. Lysine was the only limiting amino acid before or after processing. Sesame seed by-product has high-quality protein and can be a plant-based source for food formulations.

## 1. Introduction

The high demand for renewable and sustainable protein sources, the limited environmental resources, and the increased population worldwide motivate the search for alternative nutritious foods (Kumar et al., 2021; Yuliarti et al., 2021). Plant protein from food by-products could substitute animal protein sources since they can meet the protein intake requirements (Sá et al., 2019, 2020). Besides, plant proteins are good alternatives with health benefits such as low saturated fatty acid content (Görgüç et al., 2019).

Often discarded or used as feedstock for animal feed and fertilizer, oilseed meals (press cakes) are the edible oil industries by-products after the oil extraction from the seeds. These residues are underestimated as protein sources for human consumption (Kotecka-Majchrzak et al., 2020; Sá et al., 2021). Besides, these oilseed meals are great sources of fatty acids and bioactive metabolites (Gahfoor et al., 2018; Kumar et al., 2021). Regardless of the promising use of oilseed meals as protein sources in the human diet, the presence of some unfavorable compounds for protein digestibility (so-called antinutritional factors), like trypsin

inhibitors, phytates, and tannins, may limit the use of some plant proteins (Sá et al., 2021). Several studies show that conventional processing techniques (e.g., cooking, microwave, fermentation, and extrusion) and emerging technologies (e.g., ultrasound, high pressure, cold plasma, and enzymatic processes) can improve the nutritional quality of plant proteins and eliminate compounds that impair protein digestibility (Pojić et al., 2018; Sá et al., 2019).

Sesame (*Sesamum indicum* L.) seed is rich in lipids (44–58%), protein (18–25%), and carbohydrate (13–20%) contents. Also, it presents significant amounts of phytosterols, polyunsaturated fatty acids, tocopherols, and lignans (e.g., phenylpropanoid compounds) (Pathak et al., 2014). Sesame seeds are often processed into sesame seed oil, roasted sesame seed, and tahini (sesame paste). In 2017, the global cultivated area of sesame crop was over 10 million ha, producing 5.90 million tons, where sesame production in Asia and Africa represents more than 93% of global production (Sharaby and Butovchenko, 2019). Based on the annual processed sesame seeds in the world, approximately 18% of the total weight is separated as industrial by-products (~1 million tons) (Görgüç et al., 2019). Since sesame seed meal contains approximately

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33% protein (Sá et al., 2021), about 330,000 tons of plant-based protein from sesame seeds by-products can be recovered annually. Studies have successfully extracted sesame seed protein from defatted seeds with useful functional properties for food applications (Guerra and Park, 1975; Idowu et al., 2021; Khalid et al., 2003; Zhao et al., 2012).

This study hypothesizes that sesame seed by-product from cold-pressing oil extraction is an alternative plant-based source with a high nutritional value protein for human nutrition and that the food processing interventions can positively influence the nutritional quality of this meal. To the best of the authors' knowledge, few incomplete data about sesame seed meal nutritional quality is available, bringing this study's novelty. Also, information concerning the protein digestibility and amino acid profile of this oilseed by-product evaluating the influence of food processing on these responses is scarce.

Therefore, this study aimed to determine the chemical composition, antinutritional factors (ANFs) content, in vitro protein digestibility (IVPD), amino acid (AA) profile, and the Amino Acid Score of sesame seed meal. Also, it evaluated cooking, microwave, and ultrasound effect on the IVPD, AA-profile and -score, ANFs, and functional properties (solubility, water- and oil-holding capacities, and foaming) to validate the potential of sesame seed by-product as a protein supply for the human diet.

## 2. Material and methods

### 2.1. Chemicals

The chemicals supplied by Anidrol® (Diadema, Brazil) were: ethanol (99.5%), ethyl ether (99.8%), and sulfuric acid. Neon® (Suzano, Brazil)

chemicals were: acetic acid, boric acid, copper sulfate II pentahydrate, dimethyl sulfoxide, hydrochloric acid, methanol, n-hexane, sodium hydroxide, thioglycolic acid, and vanillin. Sigma® (Missouri, EUA) supplied: ammonium iron (III) sulfate dodecahydrate (purity 99%), BAPNA (purity  $\geq$  98%), catechin hydrate (purity  $\geq$  96%), peptidase (porcine gastric mucosa pepsin, 3.200–4.500 units/mg protein, product no. P6887), pronase® protease (from *Streptomyces griseus*, 4,000,000 PU/g, CAS no. 9036–06–0), selenium dioxide (purity  $>$ 99%), sodium salt hydrate of phytic acid ( $\geq$  90% phosphorus), trypsin (porcine pancreatic trypsin type IX-S, 13.000–20.000 BAEE units/mg protein, product no. T0303), 2,2'-bipyridyl (purity 99%), and  $\alpha$ -chymotrypsin (bovine pancreatic chymotrypsin type II,  $\geq$  40 units/mg protein, product no. C4129).

### 2.2. Partially defatted sesame seed

White peeled sesame seed (*Sesamum indicum L.* cultivated in India) was purchased at a grocery store (Mundo Cerealista Comércio de Alimentos LTDA., São Paulo, Brazil). The sesame seed meal was produced employing cold-pressing extraction to obtain oil from the seed, without organic solvents, using an automatic oil extractor equipment (model YJ-110, EuroLume Iluminação e Decoração Eireli, Bauru, Brazil). The temperature of the oil extraction process was  $49 \pm 4$  °C. An 80-mesh sieve was used to separate the desired granulometry sample. Then, the sample was stored at  $-18$  °C for further analysis. Fig. 1 presents a flow diagram showing the experimentation methodologies performed in this study.

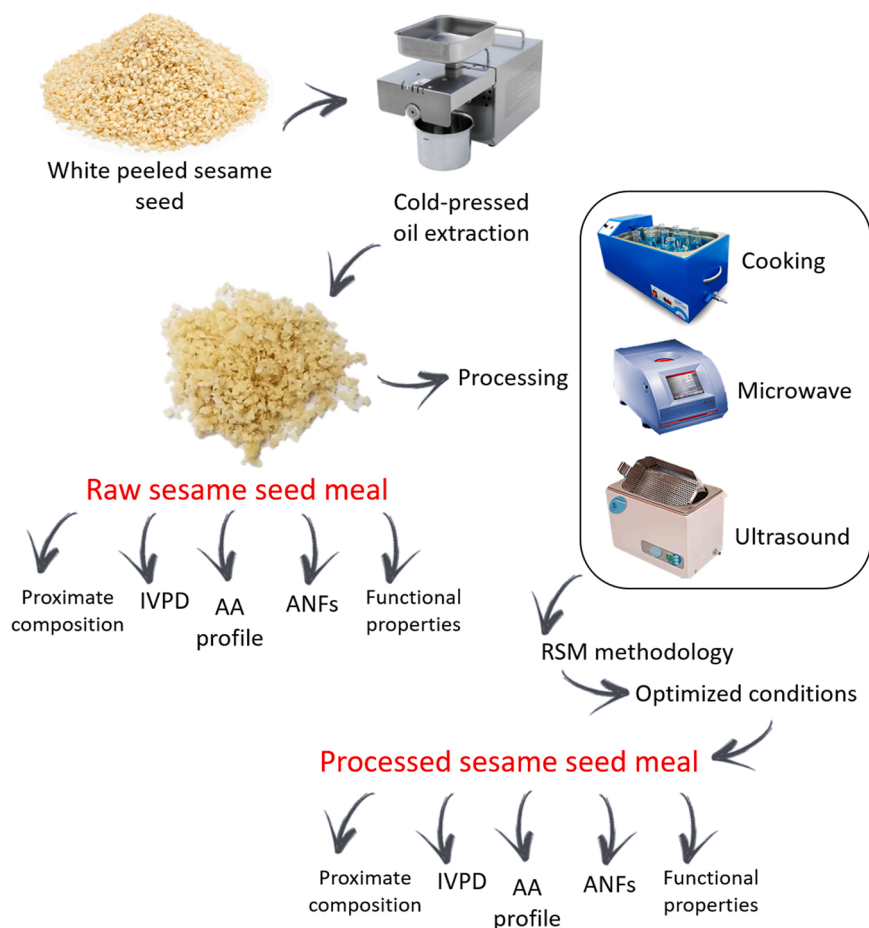


Fig. 1. A flow diagram showing the experimentation methodologies performed in this study.

### 2.3. Experimental design

Response surface methodology (RSM) and a central composite design (CCD) were used to evaluate the influence of three independent processing parameters (variables) temperature (X1), pH (X2), and time (X3) on the IVPD (dependent variable). The measured dependent variable (Y, IVPD%) fit as a function of the coded independent variables (Xi) was evaluated using a polynomial equation. Factors levels were selected according to preliminary experiments (data not shown) and based on an extensive literature review on the food processing influence on the nutritional quality of plant proteins (Sá et al., 2019).

### 2.4. Food processing

Cooking processing was conducted using water-bath equipment. Microwave processing was performed using a microwave reactor (model Monowave200, Anton Paar®, São Paulo, Brazil) working with 2.45 GHz wave frequency and operating at a maximum power of 850 W. Ultrasound processing was conducted using a bath (model USC 1400 A, Unique®, Indaiatuba, Brazil) with a frequency of 40 kHz and power density of 135 W/L. The sesame seed meal samples (protein concentration of 6.25 mg/mL, with 1:40 sample:water ratio) were processed at pre-set parameters according to the experimental CCD: temperature between 40 and 100 °C; pH values between 5.32 and 8.68; and processing time of 5 – 45 min. The protein suspension was adjusted to desirable pH with 0.1 N NaOH or 0.1 M HCl. After, the samples were freeze-dried using a laboratory freeze-dryer (model LD101, Liotop®, São Carlos, Brazil) and stored at –18 °C until further analyses.

### 2.5. Analytical methods

#### 2.5.1. Proximate composition

The proximate analysis of the raw oilseed by-products was carried out using official AOAC procedures (2012) for moisture (method 925.09), ash (923.03), lipids (920.39), nitrogen (954.01), and crude fiber (962.09), as described previously (Sá et al., 2021).

#### 2.5.2. In vitro protein digestibility (IVPD)

The Hsu et al. (1977) method with minor modifications (Tinus et al., 2012) was used to determine the IVPD of the raw and processed sesame seed meals. An enzymatic solution (trypsin,  $\alpha$ -chymotrypsin, and peptidase, pH 8.0) was added to the protein suspension (6.25 mg/mL, pH 8.0, 37 °C) at a 1:10 v/v ratio and stirred at 37 °C. After 10 min, the mixture pH was measured (pH meter model Testo 205, Testo Instrument Co., Campinas, Brazil), and IVPD was estimated according to pH variation ( $\Delta\text{pH}_{10\text{min}}$ ), as shown in Eq. 1.

$$\text{IVPD}(\%) = 65.66 + 18.10 \times \Delta\text{pH}_{10\text{min}} \quad (1)$$

#### 2.5.3. Amino acid composition

The determination of total amino acids of the sesame seed meals was performed by reverse phase column (C18 from Phenomenex®, California, EUA) chromatography in a high-performance liquid chromatograph (HPLC, Shimadzu®, Quioto, Japan) as described previously (Sá et al., 2021) using Hagen et al. (1989) and White et al. (1986) methods. Performic acid oxidation before acid hydrolysis was not employed for sulfur amino acids. Tryptophan was destroyed by acid hydrolysis and was spectrophotometrically determined (590 nm) (Spies, 1967) after enzymatic hydrolysis using pronase at 40 °C for 22 h followed by a colorimetric reaction with 4-dimethylaminobenzaldehyde (DAB) in 21.1 N sulfuric acid.

#### 2.5.4. Amino acid score and in vitro protein digestibility-correct amino acid score (IVPDCAAS)

The amino acid composition of the samples was used to estimate the Amino Acid Score (AAS) as [mg of amino acid in 1 g of test protein/mg

of amino acid in requirement pattern]  $\times$  100 (WHO/FAO/UNU Expert Consultation, 2007). The lowest AAS calculated reflects the first limiting amino acid in the protein source (Nosworthy et al., 2017), and the IVPDCAAS was calculated as a product of the AAS and IVPD values for each sample evaluated (Nosworthy et al., 2018).

#### 2.5.5. Antinutritional factors

The presence of antinutritional factors was evaluated, determining trypsin inhibition activity (TIA), tannin concentration, and phytic acid content. They were spectrophotometrically determined as described in Sá et al. (2021). The Kakade et al. (1974) method evaluated the TIA, expressed as the trypsin inhibition unit (TIU) per milligram of the sample. The tannin content was estimated by the colorimetric method of vanillin/HCl (Burns, 1971), and the concentration was expressed in mg of catechin per gram of sample. The Haug and Lantzsch (1983) method evaluated the phytic acid content, expressed as  $\mu\text{g}$  of phytate per gram of sample.

#### 2.5.6. Functional properties

**2.5.6.1. Protein solubility.** Protein solubility (PS) of the raw and processed meals were measured according to the Vogelsang-O'Dwyer et al. (2020) method with some modifications. Dispersions of 1% (w/v) of protein were prepared, and the pH was adjusted to the desired value (3 – 9) with 0.1 M HCl or 0.1 N NaOH. Then, the sample suspensions were stirred overnight at room temperature. After, these suspensions were centrifuged at maximum speed (4893g) (model K14–5000 M, KASVI®, São José do Pinhais, Brazil) for 20 min to obtain the supernatants. The supernatant protein content was measured using the Kjeldahl method (954.01, AOAC, 2012) and 6.25 as the conversion factor. PS was calculated as the protein ratio contained in the supernatant to the original sample protein content.

**2.5.6.2. Foaming capacity and stability.** The raw and processed meals foaming capacity (FC) and foam stability (FS) were determined as described by Liu et al. (2018) with minor modifications. Protein dispersions (50 mg) were prepared with 10 mL of phosphate buffer (0.01 M, pH 7) (initial liquid volume;  $V_0$ ). The sample suspensions were homogenized with an Ultra-Turrax (model T25, IKA®, Campinas, Brazil) for 2 min and poured into 50 mL graduated cylinders. The foam volume was recorded at the start ( $V_1$ ) and after 30 min ( $V_2$ ). The following equations calculated FC and FS:

$$\text{FC} (\%) = \frac{V_1 - V_0}{V_0} \times 100 \quad (2)$$

$$\text{FS} (\%) = \frac{V_2 - V_0}{V_0} \times 100 \quad (3)$$

**2.5.6.3. Water- and oil-holding capacity.** Samples water-holding capacity (WHC) and oil-holding capacity (OHC) were determined by the method of Stone et al. (2015) with some modifications. Protein suspensions (0.5 g) were mixed with soybean oil or distilled water (5 g) in a 50 mL pre-weighed centrifuge tube. Samples were vortexed for 1 min every 5 min six times and, then, centrifuged (model K14–5000 M, KASVI®, São José do Pinhais, Brazil) at maximum speed (4893g) for 15 min. The supernatant was carefully decanted, the excess oil/water in the upper phase was drained for 30 min, and the remaining samples were weighed. The WHC and OHC were determined as the percentage of water/oil absorbed per gram of sample, calculated by dividing the sample weight gained by the original weight.

### 2.6. Statistical analysis

The software Statistica® (v.13.5, Statsoft Inc., Tulsa, EUA) was used to perform the experimental data statistical analysis, adopting a

confidence level of 95% in all cases. Tukey's test compared the values of ANFs, IVPD, AA profile, AAS, and functional properties (PS, FC, FS, WHC, and OHC) of raw and processed sesame seed. These analyses were performed at least in duplicate, and results are expressed as average  $\pm$  standard deviation of replicated samples.

### 3. Results and discussions

#### 3.1. Proximate composition

Table 1 shows that sesame seed is a rich source of lipids (52.6%) and proteins (22.5%), which contributes to a high energy value (596.3 kcal/100 g). These results were similar to that reported in the literature for the same seed (Kotecka-Majchrzak et al., 2020). An increase of proteins, fibers, and available carbohydrates and a decrease of 13.9% in the lipids for sesame seed meal was observed compared to the white peeled sesame seed. The mechanical screw pressing produces more high-quality oils and meals than conventional extractions using solvent and high temperature, which leads to oil darkening and degradation of thermo-sensitive minor components (Maciel et al., 2020). This cold-pressing extraction provided 52.6% of oil yield. SSM lipids results demonstrated that a great amount of oil residual is still present in the sample, which dilutes the concentration of other nutrients in the proximate composition. The oil residual factor elimination will increase the concentration of the other constituents (protein, ash, and crude fiber). Thus, these nutrients were also calculated on a lipid-free basis and are presented in Table 1.

Furthermore, the protein result for cold-pressed SSM (35.2%) is comparable to other oilseed meals, such as cold-pressed rapeseed (32.8%) (Jia et al., 2021), black mustard seed defatted with petroleum ether (38.2%) (Sarker et al., 2015), hexane-extracted watermelon seeds (27.6%) (Lakshmi and Kaul, 2011), and cottonseed (45%) (Kumar et al., 2021; Kumar et al., 2021). The results are also comparable to traditional plant protein sources in the human diet (soybean, peas, and common beans), which present protein content of 35.3%, 21.7%, and 19.9%, respectively (Terrien, 2017). Thus, these results show the potential of the sesame seed by-product as a protein source in food formulations and human nutrition.

#### 3.2. In vitro protein digestibility (IVPD)

IVPD is a suitable tool for evaluating the nutritional quality of a food

**Table 1**  
Proximate composition and energy values of sesame seed and seed meal (raw samples).

| Proximate composition               | Sesame seed <sup>a</sup> | SSM            |
|-------------------------------------|--------------------------|----------------|
| Moisture (%)                        | 5.0                      | 4.7 $\pm$ 0.3  |
| % Dry weight basis                  |                          |                |
| Ash                                 | 4.1                      | 4.5 $\pm$ 0.1  |
| Lipids                              | 52.6                     | 45.3 $\pm$ 0.4 |
| Protein <sup>b</sup>                | 22.5                     | 35.2 $\pm$ 0.7 |
| Crude fiber                         | 2.0                      | 4.5 $\pm$ 0.9  |
| Available carbohydrate <sup>c</sup> | 8.2                      | 10.5           |
| Energy (kcal/100 g)                 | 596.3                    | 590.5          |
| % Dry weight and lipid-free basis   |                          |                |
| Ash                                 | 8.6                      | 8.2            |
| Protein <sup>1</sup>                | 47.5                     | 64.4           |
| Crude fiber                         | 4.2                      | 8.2            |
| Available carbohydrate              | 17.3                     | 19.2           |

All values are means  $\pm$  standard deviation (performed in duplicate).

SSM = sesame seed meal.

<sup>a</sup> Information provided by the seed manufacturer.

<sup>b</sup> N x 6.25.

<sup>c</sup> The available carbohydrate content was determined by calculating the percentile difference from all the other constituents according to the formula: [100 g dry weight - (g crude protein + g lipids + g ash + g crude fiber)].

protein, combined with the amino acid composition and its bioavailability (Sá et al., 2019). IVPD was 88.9  $\pm$  1.5% for the unprocessed sesame seed meal without pH adjustment (pH 6.90). Thus, the process parameters – temperature, pH, and processing time – were evaluated in the IVPD response for cooking, microwave and ultrasound processes, using an experimental design. The results obtained after 17 trials for each process are shown in Table 2. Coefficients of adjusted polynomial models and their analysis of variance (ANOVA) results were calculated and are shown in Table 3. The regression equations demonstrate an empirical relationship between the in vitro protein digestibility and the studied variables in coded units. Responses surfaces plots of independent variables on IVPD are presented in the Supplementary Materials (Figs. S1, S2, and S3). Overall, the regression model developed after ANOVA was significant ( $p < 0.05$ ) for all treatments with an insignificant lack of fit, confirming that the developed model could adequately represent the relationship among the chosen parameters.

Results for sesame seed meals processing indicated that all independent linear factors (temperature, pH, and time) significantly ( $p < 0.05$ ) affected the IVPD for ultrasound and cooking processes. However, the processing time did not significantly affect the IVPD ( $p < 0.05$ ) for the microwave. Besides, the coefficient of correlation (R) for all responses was higher than 0.93, which imply the adequacy of the applied regression models. IVPD results ranged between 87.3% and 95.3% for sesame seed meals, and the best parameters among all studied processes were 87.8°C, pH 8.00, and process time of 37 min

As seen in Figs. S1 and S3, regarding the mutual effects of the independent variables on the responses for cooking and ultrasound processing, the IVPD increased as temperature, time and pH values increased. Fig. S2 showed that IVPD was highly dependent on the pH values and temperature for microwave processing, reaching the highest response at high-temperature levels and low dependent on the processing time, which can be maintained at low levels for industrial economic viability.

As the samples were subjected to processing, a change in the conformation of sesame protein would reduce its susceptibility to the digestive enzymes, but as the processing time increased, the protein would denature, and digestion would proceed as desired (Vagadia et al., 2018). Several researchers have proposed that the treatment temperature is the key determinant of food protein digestibility. As used in this work (87.8 °C), a relatively high temperature improves protein quality while inactivating the compounds that lower the protein digestibility of plant proteins (ANFs) (Sá et al., 2019). The proper heat process can affect the conformational properties of food proteins (tertiary and secondary structure) and accelerate their denaturation without changing their primary structure or reducing protein solubility. During the hydrolytic process, the protein molecules unfold and become more accessible to proteases than in their native state (Li et al., 2010), impacting (positively or not) the protein digestibility and the amino acid profile.

Several authors demonstrated that thermal processing reduces and inactivates antinutritional factors, such as protease inhibitors, tannins, and phytic acid (Sá et al., 2019). Furthermore, high temperatures synergy with ultrasound processing can enhance mass transfer, providing high shear forces in the food matrix, modifying proteins by affecting H-bonds, reducing protein aggregates, and improving protein functionality (Görgüç et al., 2019). The processing impact on the amino acid profile, ANFs concentration, and functional properties of processed sesame seed meals is presented and discussed in Sections 3.3, 3.4, and 3.5. Additionally, according to the experimental design, pH 8.0 was the best parameter value. The results of protein solubility (pH-dependent) for the processed sesame seed meals, as discussed in Section 3.5, can help explain the influence of pH in the increasing IVPD.

The main effect of all studied processes over the sesame seed protein was the leaching out of the unfavorable compounds and the destruction of protease inhibitors, improving the protein digestibility. For the microwave process, the radiation energy can also disrupt hydrogen bonds and enable the migration of dissolved ions, affecting the secondary



**Table 2**

Experimental central composite 2<sup>3</sup>-factorial design matrix and in vitro protein digestibility responses for cooking, microwave, and ultrasound treatments of sesame seed meals (SSM).

| Run | x1, Temperature (°C) | x2, pH        | x3, Time (min) | IVPD% Cooking | IVPD% Microwave | IVPD% Ultrasound |
|-----|----------------------|---------------|----------------|---------------|-----------------|------------------|
| 1   | -1 (52.2)            | -1 (6.00)     | -1 (13)        | 87.56         | 88.29           | 87.65            |
| 2   | -1 (52.2)            | -1 (6.00)     | +1 (37)        | 88.00         | 88.83           | 88.74            |
| 3   | -1 (52.2)            | +1 (8.00)     | -1 (13)        | 91.18         | 91.00           | 91.91            |
| 4   | -1 (52.2)            | +1 (8.00)     | +1 (37)        | 92.90         | 93.17           | 92.81            |
| 5   | +1 (87.8)            | -1 (6.00)     | -1 (13)        | 90.10         | 89.73           | 90.46            |
| 6   | +1 (87.8)            | -1 (6.00)     | +1 (37)        | 90.64         | 90.10           | 90.91            |
| 7   | +1 (87.8)            | +1 (8.00)     | -1 (13)        | 94.08         | 93.90           | 93.17            |
| 8   | +1 (87.8)            | +1 (8.00)     | +1 (37)        | 95.25         | 94.44           | 94.71            |
| 9   | -1.682 (40.0)        | 0 (7.00)      | 0 (25)         | 87.29         | 87.56           | 87.74            |
| 10  | +1.682 (100.0)       | 0 (7.00)      | 0 (25)         | 92.45         | 92.63           | 92.54            |
| 11  | 0 (70.0)             | -1.682 (5.32) | 0 (25)         | 87.56         | 87.38           | 87.83            |
| 12  | 0 (70.0)             | +1.682 (8.68) | 0 (25)         | 91.91         | 92.81           | 91.80            |
| 13  | 0 (70.0)             | 0 (7.00)      | -1.682 (5)     | 88.47         | 88.83           | 88.74            |
| 14  | 0 (70.0)             | 0 (7.00)      | +1.682 (45)    | 90.00         | 89.91           | 90.64            |
| 15  | 0 (70.0)             | 0 (7.00)      | 0 (25)         | 90.82         | 90.64           | 90.69            |
| 16  | 0 (70.0)             | 0 (7.00)      | 0 (25)         | 90.46         | 91.00           | 90.71            |
| 17  | 0 (70.0)             | 0 (7.00)      | 0 (25)         | 90.10         | 91.54           | 90.42            |

**Table 3**

Variance analysis (ANOVA) of processes parameters (temperature, pH, and time) on IVPD of sesame seed meals.

| Processes parameters | Degree of freedom | Sum of squares | Mean sum of squares | F-value | p-value | Significance | Coefficient of correlation (R) | Model  |
|----------------------|-------------------|----------------|---------------------|---------|---------|--------------|--------------------------------|--|
| <b>Ultrasound</b>    |                   |                |                     |         |         |              | 0.93                           | $IVPD\% = 90.51 + 1.19x_1 + 1.57x_2 + 0.53x_3$ |
| (1) Temperature (L.) | 19.24             | 1              | 19.24               | 731.23  | 0.0014  | S            |                                |  |
| (1) Temperature (Q.) | 0.28              | 1              | 0.28                | 10.73   | 0.0819  | NS           |                                |  |
| (2) pH (L.)          | 33.87             | 1              | 33.87               | 1287.11 | 0.0008  | S            |                                |  |
| (2) pH (Q.)          | 0.02              | 1              | 0.02                | 0.79    | 0.4670  | NS           |                                |  |
| (3) Time (L.)        | 3.77              | 1              | 3.77                | 143.36  | 0.0069  | S            |                                |  |
| (3) Time (Q.)        | 0.00              | 1              | 0.00                | 0.00    | 0.9745  | NS           |                                |  |
| 1 L by 2 L           | 0.41              | 1              | 0.41                | 15.56   | 0.0587  | NS           |                                |  |
| 1 L by 3 L           | 0.00              | 1              | 0.00                | 0.00    | 1.0000  | NS           |                                |  |
| 2 L by 3 L           | 0.10              | 1              | 0.10                | 3.89    | 0.1873  | NS           |                                |  |
| <b>Microwave</b>     |                   |                |                     |         |         |              | 0.94                           | $IVPD\% = 90.97 + 1.13x_1 + 1.81x_2$           |
| (1) Temperature (L.) | 17.37             | 1              | 17.37               | 83.71   | 0.0117  | S            |                                |  |
| (1) Temperature (Q.) | 0.01              | 1              | 0.01                | 0.05    | 0.8436  | NS           |                                |  |
| (2) pH (L.)          | 44.67             | 1              | 44.67               | 215.27  | 0.0046  | S            |                                |  |
| (2) pH (Q.)          | 0.01              | 1              | 0.01                | 0.05    | 0.8436  | NS           |                                |  |
| (3) Time (L.)        | 2.17              | 1              | 2.17                | 10.47   | 0.0837  | NS           |                                |  |
| (3) Time (Q.)        | 0.92              | 1              | 0.92                | 4.46    | 0.1692  | NS           |                                |  |
| 1 L by 2 L           | 0.26              | 1              | 0.26                | 1.26    | 0.3778  | NS           |                                |  |
| 1 L by 3 L           | 0.41              | 1              | 0.41                | 1.97    | 0.2952  | NS           |                                |  |
| 2 L by 3 L           | 0.41              | 1              | 0.41                | 1.97    | 0.2952  | NS           |                                |  |
| <b>Cooking</b>       |                   |                |                     |         |         |              | 0.93                           | $IVPD\% = 90.35 + 1.40x_1 + 1.79x_2 + 0.47x_3$ |
| (1) Temperature (L.) | 26.72             | 1              | 26.72               | 203.87  | 0.0049  | S            |                                |  |
| (1) Temperature (Q.) | 0.28              | 1              | 0.28                | 2.17    | 0.2789  | NS           |                                |  |
| (2) pH (L.)          | 43.70             | 1              | 43.70               | 333.44  | 0.0030  | S            |                                |  |
| (2) pH (Q.)          | 0.14              | 1              | 0.14                | 1.05    | 0.4125  | NS           |                                |  |
| (3) Time (L.)        | 3.06              | 1              | 3.06                | 23.32   | 0.0403  | S            |                                |  |
| (3) Time (Q.)        | 0.05              | 1              | 0.05                | 0.37    | 0.6063  | NS           |                                |  |
| 1 L by 2 L           | 0.00              | 1              | 0.00                | 0.01    | 0.9501  | NS           |                                |  |
| 1 L by 3 L           | 0.02              | 1              | 0.02                | 0.18    | 0.7127  | NS           |                                |  |
| 2 L by 3 L           | 0.46              | 1              | 0.46                | 3.51    | 0.2018  | NS           |                                |  |

S – There is significant effect of process parameter on response variable (IVPD%) at 5% significant level ( $p < 0.05$ ).

NS – There is no significant effect of process parameter on response variable (IVPD%) at 5% significant level ( $p < 0.05$ ).

protein structure, improving functional properties (Section 3.5), and enhancing the IVPD. Ultrasound also accelerates the mass transfer of compounds and improves solubility due to the cellular structure's high stress and deformation, modifying the protein by affecting H-bonds, increasing protein recovery, reducing protein aggregates, and improving protein functionality (Sá et al., 2022).

Finally, the time process is a crucial parameter. Overheating proteins may depress digestibility and amino acid availability, causing a slower

release of amino acids from the protein and decomposition of essential amino acids. Therefore, a safe heating process is critical to processing plant proteins to establish maximum nutritional value (Sá et al., 2019).

### 3.3. Amino acid composition, amino acid score, and in vitro protein digestibility-correct amino acid score (IVPDCAAS)

The quality of dietary protein is imperative to human nutrition. The

potential of a protein source is determined by the amino acid profile, protein digestibility, and bioavailability to provide adequate human nutrition (Kumar et al., 2022). The amino acid (AA) composition of the raw and processed samples of sesame seed meals is presented in Table 4. The total AA content for the SSM raw sample shows similarity to those results presented for protein content (Table 1), which corroborates the analysis's veracity, and these findings agree with previous studies and literature review (Sá et al., 2020, 2021), reporting the amino acid profile of oilseeds sources and by-products.

The SSM raw sample presented statistical differences ( $p < 0.05$ ) between all processing treatments for each amino acid evaluated, except tryptophan (in cooking). Essential amino acids (EAA) and total AA content were reduced for cooking (5% and 3%, respectively), microwave (4% and 12%), and ultrasound (7% and 6%) when compared to the raw sample. The results showed that the major reductions in amino acid concentration regarding all processing were valine (~30%), leucine (~29%), aromatic amino acids (Phe + Tyr, ~28%), isoleucine (~28%), lysine (~28%), and alanine (~28%). Studies showed that thermal treatments – such as cooking (100–120 °C, 50–90 min) and microwave

(15 min) – can decrease the amino acid concentration for chickpea, regarding lysine, tryptophan, arginine, total aromatic and sulfur-containing amino acids (Alajaji and El-Adawy, 2006; Clemente et al., 1998).

Although all processing techniques showed a slight decrease in amino acid concentration, the results for the total essential amino acids (EAA) and non-essential amino acids (NEAA) are excellent for this alternative protein source. This behaviour was also observed when verifying the Amino Acid Score (Table 4). Following the requirement pattern of EAA (WHO/FAO/UNU Expert Consultation, 2007), the only limiting amino acid for all SSM samples was lysine. These are very promising results as they demonstrate that even with reducing the amino acid composition when submitting the samples in cooking, microwave and ultrasound processing, this decrease was not significant for the amino acid score, except for lysine. Some interventions can be made to guarantee the proper lysine consumption, according to the requirement pattern: a) increasing the daily intake of this protein source (~125 g of SSM raw sample); and b) supplementing the diet with plant proteins that are rich in lysine, such as chickpeas, soybeans, and peas, as

Table 4

Amino acid composition, Amino Acid Score for adults, and IVPDCAAS of the raw and processed sesame seed meals.

| AA composition (g/100 g protein)              | Requirement pattern <sup>1</sup> (g/100 g protein) | Raw Sample                   |                             | Cooking                       |                             | Microwave                    |                             | Ultrasound                   |                              |
|---|--|------------------------------|-----------------------------|-------------------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|------------------------------|
|   |  | AA                           | AAS <sup>2</sup> (%)        | AA                            | AAS <sup>2</sup> (%)        | AA                           | AAS <sup>2</sup> (%)        | AA                           | AAS <sup>2</sup> (%)         |
| <b>Essential (EAA)</b>                        |  |                              |                             |                               |                             |                              |                             |                              |                              |
| Histidine (His)                               | 1.5  | 3.46<br>± 0.01 <sup>C</sup>  | 230.9<br>± 0.1 <sup>d</sup> | 2.65<br>± 0.01 <sup>A</sup>   | 176.3<br>± 0.1 <sup>a</sup> | 2.71<br>± 0.01 <sup>B</sup>  | 180.4<br>± 0.1 <sup>c</sup> | 2.65<br>± 0.01 <sup>A</sup>  | 176.9<br>± 0.5 <sup>b</sup>  |
| Isoleucine (Ile)                              | 3.0  | 5.92<br>± 0.01 <sup>C</sup>  | 197.2<br>± 0.2 <sup>c</sup> | 4.30<br>± 0.03 <sup>A</sup>   | 143.4<br>± 1.0 <sup>a</sup> | 4.43<br>± 0.10 <sup>B</sup>  | 147.5<br>± 3.2 <sup>b</sup> | 4.25<br>± 0.04 <sup>A</sup>  | 141.6<br>± 1.4 <sup>a</sup>  |
| Leucine (Leu)                                 | 5.9  | 9.04<br>± 0.02 <sup>C</sup>  | 153.2<br>± 0.3 <sup>c</sup> | 6.33<br>± 0.02 <sup>A</sup>   | 107.4<br>± 0.3 <sup>a</sup> | 6.42<br>± 0.01 <sup>B</sup>  | 108.9<br>± 0.2 <sup>b</sup> | 6.37<br>± 0.04 <sup>A</sup>  | 108.0<br>± 0.6 <sup>a</sup>  |
| Lysine (Lys)                                  | 4.5  | 3.59<br>± 0.01 <sup>C</sup>  | 79.7<br>± 0.3 <sup>c</sup>  | 2.62<br>± 0.01 <sup>B</sup>   | 58.2<br>± 0.2 <sup>b</sup>  | 2.62<br>± 0.03 <sup>B</sup>  | 58.1<br>± 0.6 <sup>b</sup>  | 2.45<br>± 0.03 <sup>A</sup>  | 54.4<br>± 0.7 <sup>a</sup>   |
| Threonine (Thr)                               | 2.3  | 5.04<br>± 0.07 <sup>C</sup>  | 219.0<br>± 3.1 <sup>c</sup> | 3.79<br>± 0.08 <sup>B</sup>   | 164.8<br>± 3.5 <sup>b</sup> | 3.68<br>± 0.01 <sup>A</sup>  | 159.9<br>± 0.1 <sup>a</sup> | 3.75<br>± 0.01 <sup>AB</sup> | 163.1<br>± 0.6 <sup>ab</sup> |
| Tryptophan (Trp)                              | 0.6  | 1.23<br>± 0.03 <sup>C</sup>  | 204.5<br>± 6.0 <sup>c</sup> | 1.25<br>± 0.02 <sup>C</sup>   | 208.4<br>± 3.9 <sup>c</sup> | 1.06<br>± 0.03 <sup>B</sup>  | 176.4<br>± 4.7 <sup>b</sup> | 0.83<br>± 0.03 <sup>A</sup>  | 137.6<br>± 1.5 <sup>a</sup>  |
| Valine (Val)                                  | 3.9  | 6.91<br>± 0.02 <sup>D</sup>  | 177.2<br>± 0.4 <sup>d</sup> | 4.81<br>± 0.01 <sup>B</sup>   | 123.3<br>± 0.3 <sup>b</sup> | 4.91<br>± 0.01 <sup>C</sup>  | 125.8<br>± 0.3 <sup>c</sup> | 4.75<br>± 0.01 <sup>A</sup>  | 121.8<br>± 0.4 <sup>a</sup>  |
| Total sulfur amino acids (Met + Cys)          | 2.2  | 3.37<br>± 0.03 <sup>D</sup>  | 153.3<br>± 1.6 <sup>d</sup> | 3.23<br>± 0.02 <sup>B</sup>   | 146.9<br>± 0.8 <sup>b</sup> | 3.30<br>± 0.03 <sup>C</sup>  | 149.8<br>± 1.4 <sup>c</sup> | 3.09<br>± 0.01 <sup>A</sup>  | 140.4<br>± 0.4 <sup>a</sup>  |
| Total aromatic amino acids (Phe + Tyr)        | 3.8  | 11.84<br>± 0.01 <sup>D</sup> | 311.7<br>± 0.1 <sup>d</sup> | 8.52<br>± 0.01 <sup>B</sup>   | 224.3<br>± 0.3 <sup>b</sup> | 8.57<br>± 0.01 <sup>C</sup>  | 225.6<br>± 0.3 <sup>c</sup> | 8.45<br>± 0.04 <sup>A</sup>  | 222.5<br>± 1.1 <sup>a</sup>  |
| <b>Non-essential (NEAA)</b>                   |  |                              |                             |                               |                             |                              |                             |                              |                              |
| Alanine (Ala)                                 | –  | 6.38<br>± 0.03 <sup>C</sup>  | –                           | 4.54<br>± 0.02 <sup>A</sup>   | –                           | 4.57<br>± 0.01 <sup>A</sup>  | –                           | 4.66<br>± 0.02 <sup>B</sup>  | –                            |
| Arginine (Arg)                                | –  | 20.25<br>± 0.17 <sup>C</sup> | –                           | 15.39<br>± 0.05 <sup>AB</sup> | –                           | 15.26<br>± 0.08 <sup>A</sup> | –                           | 15.56<br>± 0.01 <sup>B</sup> | –                            |
| Aspartic acid (Asp)                           | –  | 9.54<br>± 0.02 <sup>B</sup>  | –                           | 7.88<br>± 0.04 <sup>A</sup>   | –                           | 7.85<br>± 0.01 <sup>A</sup>  | –                           | 7.90<br>± 0.03 <sup>A</sup>  | –                            |
| Glutamic acid (Glu)                           | –  | 23.61<br>± 0.12 <sup>C</sup> | –                           | 20.74<br>± 0.02 <sup>A</sup>  | –                           | 20.70<br>± 0.03 <sup>A</sup> | –                           | 21.03<br>± 0.01 <sup>B</sup> | –                            |
| Glycine (Gly)                                 | –  | 7.09<br>± 0.01 <sup>C</sup>  | –                           | 5.16<br>± 0.03 <sup>A</sup>   | –                           | 5.15<br>± 0.03 <sup>A</sup>  | –                           | 5.24<br>± 0.01 <sup>B</sup>  | –                            |
| Proline (Pro)                                 | –  | 4.86<br>± 0.02 <sup>D</sup>  | –                           | 3.92<br>± 0.02 <sup>A</sup>   | –                           | 3.99<br>± 0.04 <sup>B</sup>  | –                           | 4.10<br>± 0.03 <sup>C</sup>  | –                            |
| Serine (Ser)                                  | –  | 6.46<br>± 0.01 <sup>D</sup>  | –                           | 4.86<br>± 0.02 <sup>B</sup>   | –                           | 4.80<br>± 0.02 <sup>A</sup>  | –                           | 4.93<br>± 0.04 <sup>C</sup>  | –                            |
| <b>Total EAA (g/100 g protein)</b>            | –  | 39.27<br>± 0.13 <sup>C</sup> | –                           | 37.51<br>± 0.04 <sup>B</sup>  | –                           | 37.68<br>± 0.22 <sup>B</sup> | –                           | 36.59<br>± 0.03 <sup>A</sup> | –                            |
| <b>Total NEAA (g/100 g protein)</b>           | –  | 60.73<br>± 0.13 <sup>A</sup> | –                           | 62.49<br>± 0.04 <sup>B</sup>  | –                           | 62.32<br>± 0.22 <sup>B</sup> | –                           | 63.41<br>± 0.03 <sup>C</sup> | –                            |
| <b>Total AA (g/100 g sample)</b>              | –  | 36.86<br>± 0.08 <sup>D</sup> | –                           | 35.79<br>± 0.07 <sup>C</sup>  | –                           | 32.42<br>± 0.04 <sup>A</sup> | –                           | 34.53<br>± 0.04 <sup>B</sup> | –                            |
| <b>First limiting amino acid IVPDCAAS (%)</b> | –  | –                            | Lys<br>70.9                 | –                             | Lys<br>55.4                 | –                            | Lys<br>54.9                 | –                            | Lys<br>51.5                  |

All values are means ± standard deviation. Processes were performed at 87.8 °C, pH 8.0, and 37 min of time.

AA: amino acid. AAS: amino acid score. SSM: sesame seed meal. IVPDCAAS: In Vitro Protein Digestibility-Corrected Amino Acid Score.

<sup>1</sup>WHO/FAO/UNU (2007) Expert Consultation Report for adults.

<sup>2</sup>Amino Acid Score: (mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern) × 100.

<sup>A-D</sup> Different letters in the same row indicate a significant difference of the AA composition between samples for each amino acid ( $p < 0.05$  by Tukey's test).

<sup>a-d</sup> Different letters in the same row indicate a significant difference of the AAS between samples for each essential amino acid ( $p < 0.05$  by Tukey's test).

demonstrated by Sá et al. (2020).

Concerning the *in vitro* protein digestibility-correct amino acid score (IVPDCAAS), some authors suggested that this approach could be used as an alternate method for assaying protein quality that does not rely on animal experimentation (Nosworthy et al., 2017). IVPDCAAS results are shown in Table 4 for the raw and processed SSM samples. Regarding lysine as the limiting amino acid, the cooking (55.4%), microwave (54.9%), and ultrasound (51.5%) processing were not beneficial to increase the IVPDCAAS compared to the SSM raw sample (70.9%). These processes reduced the lysine concentration and directly impacted the *in vitro* protein digestibility-correct amino acid score. These findings agree with Nosworthy et al. (2018), reporting IVPDCAAS for cooked red (53.4%) and green lentils (51.4%). Thus, if only considering the IVPDCAAS, all studied processing can be unnecessary interventions to increase the protein quality of sesame seed meal. However, concerning other important properties for food applications in the industry, these processes can increase some protein functionalities, as discussed in Section 3.5.

Although a few researchers are reporting the impact of thermal processing on plant protein's amino acid profile, insufficient studies evaluate the amino acid composition of plant proteins when using emerging technologies, such as ultrasound. Information about the nutrition quality of sesame seed meals is also scarce. Besides, no data is presented in the literature concerning the influence of thermal processing and ultrasound in the protein quality of sesame seed meals, which brings the novelty of this study. Finally, there is a constant requirement for protein quality and amino acid availability, which are critical aspects of meeting human nutritional needs in a scenario of constrained environmental resources and increasing the world's population, which are scientific and technological challenges that should be addressed shortly.

### 3.4. Antinutritional factors (ANFs)

The presence of compounds considered antinutritional factors, such as proteases (i.e., trypsin) inhibitors, phytates, fibers, and tannins, in food by-products from plant origin are unfavorable for protein digestion. Phytates and tannins influence the minerals' bioaccessibility, and trypsin inhibitors hinder protein absorption by binding with proteases, as the term indicates. Although these compounds are naturally synthesized due to plant physiology, at the beginning of seed formation, the plant healing process, or during maturation, they must be removed to increase protein digestibility (Sá et al., 2021). The ANFs concentrations, regarding trypsin inhibition activity, tannin, and phytic acid, are shown in Table 5.

All sesame seed by-products did not present tannins in the analysis performed since they were prepared from dehulled/peeled seed. The raw sesame by-product showed trypsin inhibitor activity (45.9 TIU/mg) in the same order of magnitude of traditional plant protein sources (e.g., soybean: 41.5 TIU/mg) (Samaranayaka, 2017). Trypsin inhibitors are usually heat-stable and can require a long processing time for their inactivation (Vagadia et al., 2018). The samples processed by cooking, microwave, and ultrasound (87.8 °C, pH 8.0, and 37 min) have trypsin inhibitor activity efficiently reduced by 55%, 53%, and 47%, respectively.

Usually, a residual TIA of 5–20% remains after typical commercial treatments. As the name indicates, TIA impairs trypsin and chymotrypsin action, stimulates their secretion, causes hypertrophy and hyperplasia of the pancreas, and leads to adenomas and carcinomas in the exocrine pancreas. Generally, the risk assessment on TIA would include 2-year bioassays from animals treated with TI and fed diets carefully controlled. The effects of TIA on protein nutrition would have to be considered when identifying the maximum tolerated dose (Hatchcock, 1991). To the best authors' knowledge, only considering the TIA results of the sesame samples presented in this study cannot affirm if they are safe for human/animal consumption, even with or without additional

**Table 5**

Antinutritional factors concentration and functional properties of sesame seed meals.

| Analyses                              | Raw Sample                  | Cooking                     | Microwave                   | Ultrasound                   |
|---------------------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------|
| <b>Antinutritional factors (ANFs)</b> |                             |                             |                             |                              |
| TIA (TIU/mg sample)                   | 45.9<br>± 0.9 <sup>C</sup>  | 24.5<br>± 0.6 <sup>B</sup>  | 21.5<br>± 0.6 <sup>A</sup>  | 20.8<br>± 0.8 <sup>A</sup>   |
| Phytic acid (µg/g sample)             | 26.1<br>± 0.2 <sup>D</sup>  | 4.9<br>± 0.7 <sup>A</sup>   | 17.3<br>± 0.4 <sup>C</sup>  | 7.5 ± 0.3 <sup>B</sup>       |
| Tannin (mg catechin/g sample)         | n.d. <sup>A</sup>           | n.d. <sup>A</sup>           | n.d. <sup>A</sup>           | n.d. <sup>A</sup>            |
| <b>Functional properties</b>          |                             |                             |                             |                              |
| Foaming capacity (%)                  | 51.9<br>± 0.6 <sup>A</sup>  | 96.9<br>± 0.9 <sup>C</sup>  | 94.8 ± 0.4 <sup>B</sup>     | 94.3 ± 0.4 <sup>B</sup>      |
| Foaming stability (%)                 | 15.3<br>± 0.4 <sup>A</sup>  | 49.4<br>± 0.9 <sup>D</sup>  | 45.6<br>± 0.9 <sup>C</sup>  | 40.3 ± 0.4 <sup>B</sup>      |
| Water-holding capacity (g/g)          | 1.73<br>± 0.08 <sup>A</sup> | 2.01<br>± 0.01 <sup>B</sup> | 2.06<br>± 0.01 <sup>B</sup> | 1.99<br>± 0.02 <sup>B</sup>  |
| Oil-holding capacity (g/g)            | 0.93<br>± 0.04 <sup>A</sup> | 1.03<br>± 0.01 <sup>B</sup> | 1.10<br>± 0.01 <sup>C</sup> | 1.06<br>± 0.02 <sup>BC</sup> |

All values are means ± standard deviation.

Processes were performed at 87.8 °C, pH 8.0, and 37 min of time.

n.d.: not detected; SSM: sesame seed meal. TIA: trypsin inhibitor activity. TIU: trypsin inhibitor unit.

<sup>A-C</sup> Different letters in the same row indicate a significant difference between samples for each analysis ( $p < 0.05$  by Tukey's test).

heating, without an extended and specific risk assessment.

The highest level of phytic acid was noticed in the raw sesame seed meal sample (0.0026 g/100 g), which is extremely lower than traditional sources of plant proteins (e.g., pea: 1.2; soybean: 2.0; chickpea: 1.5; common bean: 1.6; and rice: 0.7 g/100 g) (Sá et al., 2021). The low phytic acid content may be an oil extraction consequence that changes the chemical composition due to chemical affinity, highlighting the importance of the oil extraction step on improving protein digestion by reducing this ANF. However, cooking, microwave, and ultrasound treatments further reduced phytic acid levels for the sesame seed meals by 71%, 34%, and 81%, respectively. The decrease in the ANFs concentration performed by thermal processing and ultrasound is compatible with the increase in IVPD results presented by the experimental design (Table 2).

Few studies evaluate the ANFs concentration of oilseed by-products and plant proteins when using emerging technologies, such as ultrasound. Besides, thermal processing and ultrasound influence on the ANFs concentration of sesame seed meals are also scarce. Therefore, the antinutritional factors evaluated in terms of the content of phytic acid, tannins, and trypsin inhibitor activity indicated raw and processed SSM samples as promising protein sources for humans.

### 3.5. Functional properties

Plant-based proteins can have countless industrial applications, such as food supplements, bioactive peptides, edible food coatings/films, stabilizers or emulsifiers, adhesives, and hydrogels (Kumar et al., 2022). Protein functionality has critical importance in defining the applicability of plant proteins flours, concentrates, and isolates, which affect the physicochemical characteristics of food products (texture, appearance, stability, cohesion-adhesion, elasticity, and viscosity). Intrinsic and extrinsic factors (e.g., protein structure, amino acid composition, hydrophobicity, medium pH, salts, temperature, pressure, and ionic strength) can influence the functional properties of protein-containing foods (Gençdağ et al., 2020; Kumar et al., 2022). Protein extraction and processing may change those functional properties; thus, studying the process parameters is essential to understand the impact on food products' functional and physicochemical properties.

It is worth mentioning that the SSM samples used to conduct the

protein techno-functional properties assays were not sesame seed protein isolates. These analyses were conducted to obtain responses regarding the functionalities behaviours of the protein inserted in the plant matrix. Other compounds present in the matrix (lipids, carbohydrates, fibers, and others) can greatly influence these functional results. As presented in Table 1, SSM lipids results demonstrated that a great part of the oil is still present in the sample. This high oil concentration is an important consideration concerning the functional properties results obtained in the following sections.

### 3.5.1. Protein solubility in the plant matrix

The protein solubility is highly influenced by hydrophilicity/hydrophobicity balance, which depends on the amino acid composition, particularly at the protein surface. Higher solubility is related to a low number of hydrophobic residues, elevated net charge and the electrostatic repulsion and ionic hydration occurring at pH above and below the isoelectric pH (pI) (Onsaard, 2012).

PS of SSM samples as a function of pH is shown in Fig. 2. Similar results have been described previously in the literature for sesame seeds, but the solubility profile of sesame seed protein is remarkably different in various salt solutions (Capellini et al., 2019; Khalid et al., 2003).

The minimum protein solubility was for SSM raw sample (15%) at pH 3. The higher protein solubilization is observed at alkaline pH values (8–9), up to 70%, for raw and processed samples, which is important for food formulation. The utilization of plant proteins as ingredients for the food industry is usually limited due to their extremely low solubility at neutral pH. However, the protein solubility in the plant matrix of the processed sesame seed meals presented here demonstrated promising results for applications in the food industry.

The high solubility of SSM samples at pH 8 may have also influenced the increase in IVPD when the processing treatments occurred at this pH value, as discussed in Section 3.2, due to the sesame seed protein being soluble and more available in the reaction medium for the digestive enzymes attack, simulated in the in vitro digestibility analysis.

### 3.5.2. Foaming capacity and stability in the plant matrix

Protein foaming agents should stabilize foams rapidly and effectively at low concentrations and perform as an effective foaming agent over the pH range and in the medium with foam inhibitors (e.g., fat, alcohol, or flavor substances) (Zayas, 1997). FC and FS of raw and processed SSM samples are presented in Table 5. Foaming properties of SSM raw samples were analyzed at their original pH (6.9), while for processed samples, the properties were evaluated at pH 8, which was the best

processing condition found for IVPD.

Although the raw sample presented an inferior foaming capacity (51.9%), cooking, microwave, and ultrasound increased FC by 87%, 83%, and 82%, respectively, with FC up to 97%. A similar result (100%) has been described previously by Khalid et al. (2003) for sesame seed protein isolate at the same pH conditions. This behaviour was likely due to the increased net charges on the protein, weakening the hydrophobic interactions, increasing the protein flexibility, and allowing the protein to diffuse more rapidly to the air-water interface to encapsulate air particles, which enhances the foam formation (Khalid et al., 2003).

The best protein foaming agents in the food industry are egg white, gelatins, casein, soybean proteins, and gluten (Zayas, 1997). These good FC results for SSM samples demonstrated viability for their applications in the food industry due to plant proteins with foaming properties being good for salad dressings and soups (Onsaard, 2012).

The foaming stability was 15% for the raw sesame seed meal, while SSM processed by cooking, microwave, and ultrasound increased FS by 223%, 198%, and 163%, respectively, with results up to 50%. Although SSM samples foaming properties decreased after 30 min of analysis, some interventions can assure higher foaming stability. The addition of salts can significantly enhance protein FS due to increased solubility and surface activity of the soluble protein (Khalid et al., 2003).

### 3.5.3. Water- and oil-holding capacities in the plant matrix

Water-holding capacity is mainly attributed to a protein matrix's ability (e.g., protein particles, gels, or muscle) to absorb and retain water against gravity, including bound, hydrodynamic, capillary, and physically entrapped water (Onsaard, 2012). The protein WHC is very important in meat processing, affecting the products' juiciness, tenderness, and taste (Mu et al., 2017). Oil-holding capacity refers to the oil physical entrapment, the number of nonpolar side chains on proteins, and their different conformational features that bind hydrocarbon chains on the fatty acids (Khalid et al., 2003). Proteins with good oil-holding capacities can be widely used in egg yolk products, meat products, dairy products, coffee mate, dough, and cake pastes (Mu et al., 2017). WHC and OHC of raw and processed SSM samples are presented in Table 5.

The water-holding capacity was 1.7 g H<sub>2</sub>O/g for the raw sesame seed meal, while SSM processed by cooking, microwave, and ultrasound increased 16%, 19%, and 15% of WHC, respectively, with results up to 2.1 g H<sub>2</sub>O/g. The same value (2.1 g H<sub>2</sub>O/g) was found by Khalid et al. (2003) for sesame seed protein isolate, within the range of protein concentrates commercial values (1.9 – 2.2). They suggested that

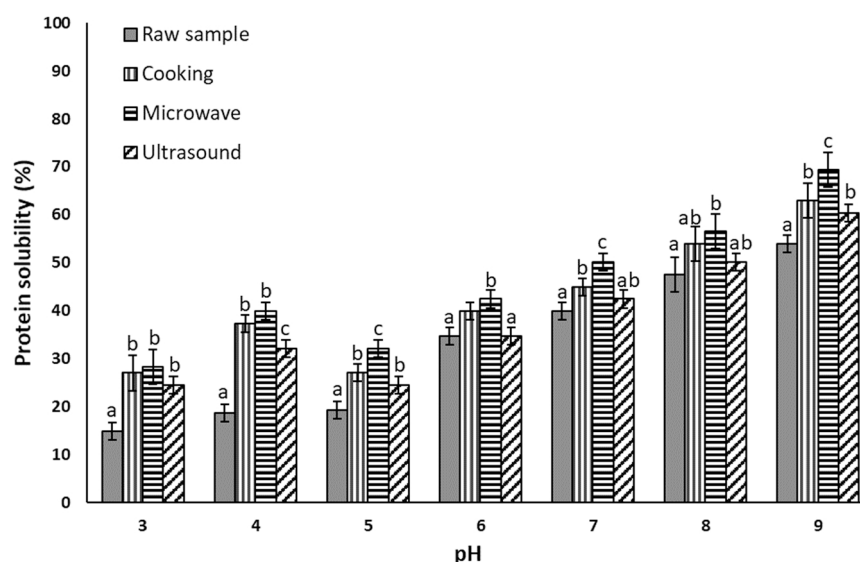


Fig. 2. Effect of pH on protein solubility in plant matrix of sesame seed meals.



carbohydrates and other components of the plant matrix may impair WHC, while protein isolates have great ability to swell, dissociate and unfold, exposing additional binding sites increasing their WHC. However, for this functionality, WHC results for processed SSM samples showed promising viability for applications in the food industry, similar to sesame seed concentrates and isolates.

The oil-holding capacity was 0.9 g oil/g for the raw sesame seed meal, while SSM processed by cooking, microwave, and ultrasound increased OHC by 11%, 18%, and 14%, respectively, with results up to 1.1 g oil/g, which was lower to results presented by previously by [Khalid et al. \(2003\)](#) for sesame seed protein isolate and soybean. These WHC and OHC results for SSM samples demonstrated viability for their applications in the food industry due to plant proteins with high oil- and water-holding capacities being desirable for use in meat ([Onsaard, 2012](#)) or meat analogues products.

#### 4. Conclusions

Cooking, microwave, and ultrasound at different conditions of temperature (40 – 100 °C), pH (5.32 – 8.68), and time (5 – 45 min) were processing techniques that impacted the raw sesame seed meal. The surface responses showed that IVPD depended on the temperature, time, and pH for cooking and ultrasound processing. However, IVPD had reached the highest response at high-temperature levels for microwave processing without depending on the processing time. Thus, this latter can be used in a shorter time, leading to industrial economic viability. Processing at 87.8 °C, pH 8.0, and 37 min increased 7% the IVPD response, from 89% up to 95%, which is a high value for a plant protein source. Similarly, ANFs have reduced: TIA declined by 55%, and phytic acid had up to 81% decrease, while tannins were not detected. Thus, these techniques can be used as potential methods of processing sesame seed by-products to increase protein digestibility and eliminate anti-nutritional factors.

Lysine was the only limiting amino acid for all sesame seed meal samples. Although cooking, microwave and ultrasound influenced the amino acid profile by reducing up to 30% valine, leucine, isoleucine, lysine, alanine, and aromatic amino acids, the composition and amino acid score brought promising results. Processing did not decrease the amino acid score for the essential amino acids, except for lysine, which directly affected the processed sesame seed meal IVPDCAAS (~55%) compared to the raw sample (~71%). Hence, if only considering the IVPDCAAS, all studied processing can be unnecessary interventions to increase the protein quality of sesame seed meal. However, concerning techno-functional properties, the protein solubility, water- and oil-holding capacity, and foaming properties in plant matrix demonstrated that processed sesame seed meals are promising and can be used as alternative protein sources aiming food formulation systems.

Based on this study reports about the influence of cooking, microwave, and ultrasound on the nutritional and functional quality, one can conclude that sesame seed by-products from the oil extraction industries are sustainable and high-quality protein sources, which can be used as technological ingredients for food formulations and may become extra income for the industry while minimizing large waste disposals and collaborating with the environment. Additionally, aiming large scale in industry practices, operating parameters and equipment configurations must be studied and thoroughly discussed.

#### CRedit authorship contribution statement

**Amanda Gomes Almeida Sá:** Conceptualization, Methodology, Investigation, Validation, Writing – original draft preparation. **Maria Teresa Bertoldo Pacheco:** Methodology, Resources. **Yara Maria Franco Moreno:** Conceptualization, Methodology, Writing – review & editing. **Bruno Augusto Mattar Carciofi:** Conceptualization, Methodology, Resources, Supervision, Project administration, Writing – review & editing.

#### 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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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jfca.2022.104634](https://doi.org/10.1016/j.jfca.2022.104634).

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