

Research Article

Effect of Atmospheric Pressure Cold Plasma Treatment on the Radical Scavenging and Antioxidant Activities, Phenolic Content, and Some Nutritional Characteristics of Chia Seeds (*Salvia hispanica* L.)

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This study investigates the potential effects of atmospheric pressure cold plasma (ACP) treatment as a novel nonthermal processing method for plant-derived products, specifically focusing on chia seeds, a rich source of nutrients. The impacts of ACP treatment (at intensities of 6.5 kV for 3 min, 6.5 kV for 8 min, 8.5 kV for 3 min, and 8.5 kV for 8 min, which are called S1, S2, S3, and S4, respectively) on radical scavenging and antioxidant activities, phenolic content, and some nutritional characteristics of chia seeds were examined and compared with the control sample (untreated sample). Results indicated that the quantities of fat, ash, crude fiber, and carbohydrate changed on the basis of ACP treatment intensity (i.e., exposure time and the voltage applied during the treatment), suggesting that the intensity and effects of plasma-activated species play a key role in this process. In terms of in vitro starch digestibility, a significant increase was observed, from 40.14% in the control to 64.99% in the sample treated for 8 min at 8.5 kV (designated as S4). A similar trend was noted for in vitro protein digestibility, which increased from 68.39% in the control to 85.54% in S4. These improvements are attributed to the depolymerization and side-chain cleavage induced by plasma-activated species during ACP treatment. Moreover, ACP treatment led to an increase in both polyphenol content and antioxidant activity. Polyphenol content rose from 41.59 mg GAE/g in the control to 64.98 mg GAE/g in S4, while antioxidant activity increased from 68.39% to 85.45%. The enhancement in antioxidant activity may also be partly due to UV radiation generated during plasma production, which can stimulate the formation of secondary metabolites. Overall, the findings demonstrate that the effects of ACP treatment on the various parameters examined—such as proximate composition, digestibility, phenolic content, and antioxidant activity—are influenced by exposure time and voltage. These variations appear to be primarily driven by the action of plasma-activated species.

Keywords: antioxidant; chia seeds; cold plasma treatment; nutritional characteristics; phenolic

1. Introduction

Chia (*Salvia hispanica*) is an edible, flowering plant belonging to the mint family (Lamiaceae). It is a gluten-free pseudocereal

that is particularly notable for its high protein content and favorable amino acid profile, making it a high-quality protein source. In addition to their nutritional value, chia seeds are rich in vitamins, minerals, and bioactive compounds, including

chlorogenic acid, caffeic acid, phytosterols, and carotenoids [1, 2]. Chia also contains a higher proportion of mucilage (approximately 5%) compared to other cereal grains, which contributes to its superior water solubility and highly hydrophilic nature. This mucilage can absorb water and form a gel-like structure, thereby mimicking certain properties of fat [3]. Due to these unique characteristics, chia has found widespread application in the food industry, available in various forms, such as whole or ground seeds, flour, dried or soaked seeds, as well as mucilage and oil extracted from the seeds [4, 5].

Nevertheless, due to the potential contamination of chia seeds and flour by various microorganisms, particularly fungi, during cultivation, harvesting, and storage, the application of effective antimicrobial processing methods is essential [6]. Thermal processing is one of the most commonly used methods, as it effectively kills microorganisms, inactivates enzymes, and extends the shelf life of food products. However, despite these advantages, thermal treatments often result in a significant reduction in the nutritional quality and freshness of food, presenting a major challenge [7]. To address this issue, new processing techniques have been introduced in recent years to minimize potential damage to food products while maintaining their quality [8].

Emerging nonthermal treatments for food processing include pulsed light, pulsed electric fields, ultrasound, high hydrostatic pressure, and cold plasma. Among these, atmospheric pressure cold plasma (ACP) technology is a promising method for microbial inactivation and extending the shelf life of food without the application of heat [9, 10]. Plasma, the fourth state of matter, consists of ionized reactive gases generated by energizing gases, such as oxygen, nitrogen, argon, helium, and neon under low pressure and power conditions. Under these conditions, the gas becomes ionized, producing various reactive species, including photons, ions, free radicals, atoms, molecules, and free electrons. These reactive species are responsible for deactivating microbial populations [11, 12]. ACP treatment also interacts with food components, such as carbohydrates, proteins, water, and phenolic compounds, by adding or removing specific functional groups [13–17].

In recent years, the impact of ACP treatment on improving the nutritional properties of various crops, such as pearl millet, wheat, peanuts, beetroot juice, and flaxseed, has been extensively studied. These studies demonstrated that plasma treatment can effectively enhance the crude fat content and hydration properties while reducing crude fiber, protein, and moisture levels in pearl millet [18]. Additionally, it was found to promote seed germination and seedling growth while enhancing total soluble solids, protein content, photosynthetic pigments, total phenolic content (TPC), antioxidant activity, enzyme activity, free amino acids, and mineral content in wheat [19]. It was also shown to decrease aflatoxin contamination in peanuts and improve the bioavailability of essential metals, including calcium (Ca), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and magnesium (Mg) [20]. Furthermore, plasma treatment increased the phenolic content in beetroot juice [21] and improved the physicochemical, functional, and antioxidant properties of flaxseed [22].

However, to the best of our knowledge, no study has yet investigated the potential effects of ACP on the nutritional properties of chia seeds. Given the importance of pseudocereals, particularly in gluten-free diets, and the increasing shift toward nonthermal processing techniques as alternatives to conventional thermal methods (which can degrade the nutritional and sensory quality of food), the aim of the present study is to evaluate the effects of ACP treatment—at different exposure times and voltages—on the phenolic content, antioxidant activity, proximate composition, and in vitro protein and starch digestibility of chia seeds.

2. Materials and Methods

2.1. Materials. Chia seeds (black variety) were purchased from a local market in Fasa, Iran. Cupric sulfate, sodium hydroxide, potassium sulfate, sulfuric acid, boric acid, bromocresol green, methyl red, petroleum ether, phosphate buffer, potassium hexacyanoferrate (III), trichloroacetic acid, Fe (III) chloride, pepsin, pancreatin, picric acid, pancreatic bile salt, amyloglucosidase, sodium carbonate, methanol, ethanol, sodium acetate buffer, hydrochloric acid, ferric chloride, α -amylase, and sodium malate buffer were obtained from Merck (Germany). The Folin–Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), and TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) were purchased from Sigma-Aldrich (United States). An enzymatic glucose oxidase–peroxidase kit was sourced from Megazyme (Ireland). All chemicals and reagents used were of analytical grade.

2.2. ACP Treatment. Plasma treatment was conducted using an atmospheric pressure plasma reactor (High Tech Company, 0045, Tehran, Iran) based on a dielectric barrier discharge (DBD) configuration. The plasma parameters, including electrode voltage, treatment time, and operating frequency, were predefined before the experiment. An operating frequency of 37.3 kHz was set as a constant parameter, while voltage and treatment time were varied during the experiment. The temperature of the air, which served as the working gas, was maintained at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Chia seeds were subjected to ACP treatment at voltages of 50 and 60 kV for durations of 5 and 10 min, respectively, and were subsequently milled. A total of five samples were prepared, including four ACP-treated samples (6.5 kV for 3 min, 6.5 kV for 8 min, 8.5 kV for 3 min, and 8.5 kV for 8 min) and one untreated control sample. All samples were then milled and passed through mesh sieves with Numbers 40 and 50 (corresponding to particle sizes of 297–400 μm). The fractions between the two sieves were collected, stored in airtight bags, and kept at -18°C for further analysis [6].

2.3. Proximate Composition of ACP-Treated Chia Flour

2.3.1. Protein and Fat Content. The crude protein content of chia flour was determined using the Kjeldahl method in accordance with American Association of Community Colleges (AACC) standards. Similarly, the crude fat content was analyzed using the Soxhlet extraction method [23].

2.3.2. Ash Content. The ash content was determined by weighing approximately 5 g of the chia flour sample (W1), which was then ignited. The sample was cooled in a desiccator for 10 min and subsequently heated at 550°C until a constant weight (W2) was achieved [24]. The ash content was calculated using the following formula:

$$\text{Ash content} = \left[\frac{(W2 - W1)}{\text{weight of sample}} \right] \times 100$$

2.3.3. Carbohydrate and Crude Fiber Content. Crude fiber content was determined following the Association of Official Analytical Chemists (AOAC) method [23]. For the calculation of carbohydrate content, 1.25 mL of phosphate buffer (0.2 M) and 1.25 mL of potassium hexacyanoferrate (III) (30 mM) were added to 0.5 mL of each sample, followed by incubation at 68°C for 20 min. Subsequently, 1.25 mL of trichloroacetic acid (0.6 M) was added to the extract, and the mixture was centrifuged at 3240 × g for 10 min. After centrifugation, 1 mL of distilled water and 0.2 mL of Fe (III) chloride were added to 1 mL of the aliquot in an Eppendorf tube, which was then left at room temperature for 15 min. Absorbance was measured at 700 nm. The carbohydrate content in the samples was calculated using the following equation, as described by Agu and Okoli [25]:

$$\text{Carbohydrate\%} = 100 - (\text{moisture\%} + \text{protein\%} + \text{fat\%} + \text{ash\%} + \text{crude fiber\%})$$

2.4. Digestibility of ACP-Treated Chia Flour

2.4.1. In Vitro Protein Digestibility. In vitro protein digestibility of chia flour was evaluated using a modified version of the Akeson and Stahmann method [26]. A pepsin-pancreatin digestion approach was employed for a rapid and accurate assessment of protein digestibility. Dried samples were mixed with 12.5 mg of pepsin and 4 mg of pancreatin in 15 mL of 0.1 N HCl and incubated in a water bath at 37°C for 24 h. The solution was then neutralized with 0.2 N NaOH. The digested mixtures were further incubated with phosphate buffer at pH 8.0 for 24 h at 37°C. Following this, 50 mL of 1% (w/v) picric acid was added to the mixture, which was then centrifuged at 1000 × g for 30 min. The supernatant was collected for amino acid analysis. These amino acids were extracted using buffers at pH 3.25 and 4.25. Released amino acids were quantified through automated amino acid analysis, enabling rapid protein quality assessments using a minimal sample of chia flour (500 mg).

2.4.2. In Vitro Starch Digestibility. In vitro starch digestibility was determined using an enzymatic glucose oxidase-peroxidase kit with slight modifications. Initially, 0.1 g of dried sample was mixed with 0.2 mL of α -amylase in 10 mL of sodium malate buffer (pH 7) and incubated in a water bath at 37°C for 24 h. Subsequently, 200 μ L of this solution was combined with 200 μ L of 96% (w/w) ethanol and centrifuged at 1000 × g for 30 min. From the resulting mixture, 100 μ L of the supernatant was collected and mixed with 850 μ L of sodium acetate buffer and 50 μ L of amyloglu-

cosidase. This solution was then incubated in a water bath at 50°C for 30 min. Glucose was used as the standard. In vitro starch digestibility was measured using the glucose oxidase-peroxidase kit and calculated based on the total starch content using the following equation [27]:

$$\text{Total starch (g)} = \text{glucose released (g)} \times 0.9 \times 100$$

2.5. TPC of ACP-Treated Chia Flour. TPC was determined using the Folin-Ciocalteu reagent. A 0.5 mg sample of chia flour was mixed with 2.5 mL of Folin-Ciocalteu reagent (10× diluted) and 2 mL of sodium carbonate (7.5% w/v). The mixture was then incubated at room temperature for 60 min. Absorbance was measured at 765 nm using a spectrophotometer (Cecile, Germany). Methanol was used as the reference standard. TPC was expressed as milligrams of gallic acid per gram of sample [28, 29].

2.6. Antioxidant Activity of ACP-Treated Chia Flour

2.6.1. DPPH Method. Antioxidant activities were evaluated using a modified version of the method by Lim, Park, and Yoon [30]. In this procedure, 0.5 mL of DPPH solution (0.2 mM in ethanol) was added to 0.5 mL of each sample extract. The mixtures were then incubated at room temperature for 30 min, and the absorbance was measured at 517 nm using a spectrophotometer (Cecile, Germany). The scavenging activity of each sample solution was compared to a control solution as follows:

$$\text{Percentage of inhibition (\%)} = \left[\frac{(A_{\text{Control}} - A_{\text{Sample}})}{A_{\text{Control}}} \right] \times 100$$

where A_{Control} and A_{Sample} were the control and sample absorption amounts, respectively.

2.6.2. FRAP (Ferric Ion Reducing Antioxidant Power). This assay is used to measure the antioxidant capacity of foods, beverages, and nutritional supplements containing polyphenols [31]. Briefly, a mixture of 300 mL/mol of acetate buffer (pH = 3.6), 10 mM/L of TPTZ (in 40 mM/L HCl), and 20 mM/L of ferric chloride (with a ratio of 10:1:1, respectively) was prepared. Then, 150 μ L of the prepared extract was mixed with 4.5 mL of FRAP reagent. After 5 min, the absorbance was measured at 593 nm using a UV/vis spectrophotometer (Cecile, Germany) and expressed as μ mol Fe²⁺/g [32].

2.6.3. Reducing Power. To measure antioxidant activity using this method, 2.5 mL of phosphate buffer and 2.5 mL of potassium hexacyanoferrate were mixed with 0.5 mL of each sample extract. The mixtures were then incubated at 50°C for 30 min. After incubation, 1.25 mL of trichloroacetic acid was added, and the mixture was centrifuged at 3240 × g for 10 min. Subsequently, 2.5 mL of the upper layer was combined with 2.5 mL of distilled water and 0.5 mL of Fe chloride. The final mixture was allowed to stand at room temperature for 15 min, after which the absorbance was measured at 700 nm using a spectrophotometer (Cecile, Germany). The results were expressed as μ mol Fe²⁺/g [33].

TABLE 1: Effect of plasma treatment on proximate content of chia flour.

Parameter	Control	S1	Treatment S2	S3	S4
Fat (%)	30.30 ± 0.97 ^c	30.83 ± 0.86 ^c	33.12 ± 0.35 ^a	30.80 ± 0.1 ^c	32.70 ± 0.1 ^b
Ash (%)	4.12 ± 0.07 ^b	4.21 ± 0.02 ^b	4.41 ± 0.03 ^a	4.40 ± 0.14 ^a	4.21 ± 0.014 ^b
Protein (%)	23.98 ± 2.00 ^b	23.65 ± 1.62 ^c	24.85 ± 1.90 ^a	24.20 ± 2.26 ^b	24.55 ± 1.48 ^a
Fiber (%)	29.80 ± 1.97 ^c	36.06 ± 1.41 ^b	27.85 ± 2.47 ^d	37.30 ± 1.83 ^a	28.30 ± 2.12 ^{cd}
Carbohydrate (%)	11.90 ± 0.56 ^a	5.10 ± 0.28 ^d	9.90 ± 0.56 ^c	3.50 ± 0.42 ^e	10.25 ± 0.21 ^b

Note: Data are reported as *average ± standard deviation*. Values followed by different letters in each row are significantly different ($p < 0.05$). Control is an untreated sample, and S1, S2, S3, and S4 are plasma-treated flours for 3 min at 6.5 kV, 8 min at 6.5 kV, 3 min at 8.5 kV, and 8 min at 8.5 kV, respectively.

2.7. Statistical Analyses. To ensure the highest accuracy in the statistical results, all relevant tests were performed in triplicate. The results were analyzed using ANOVA with SPSS software (IBM SPSS, Version 21). Tukey's test was applied for post hoc comparison to identify significant differences. Statistical significance was set at $p < 0.05$. The correlation of variables has been assessed using Pearson's correlation. The charts were generated using Excel software.

3. Results and Discussion

3.1. Nutritional Properties of ACP-Treated Chia Flour. Table 1 presents the proximate composition of fat, ash, protein, fiber, and carbohydrate in ACP-treated chia flours, compared to the control.

3.1.1. Fat Content. As shown in Table 1, a general increase in fat content is observed with ACP treatment. Among the ACP-treated samples, the highest fat content (33.12%) is found in the S2 samples (ACP-treated for 8 min at 6.5 kV). ACP treatment significantly increases fat content in relation to exposure time ($p < 0.05$). As demonstrated, no significant differences existed between S1 and S3 (i.e., 5-min ACP-treated samples at either 6.5 or 8.5 kV) and the control samples, and a more noticeable increase in fat content is seen in the samples treated for 10 min, particularly at the lower voltage (the highest fat content is found in S2). The increase in fat content due to ACP treatment may be attributed to the breakdown of crude fat into simpler derivatives, which subsequently increases the fat content [34, 35]. It is important to note that changes in the fat profile could also result from radicals (such as hydroxyl and superoxide) generated during the ACP process [18]. Studies have reported that these radicals, especially hydroxyl and superoxide anions, may alter the molecular characteristics of fats [36]. The increase in fat content during ACP treatment could also be linked to a reduction in moisture content, which, in turn, could lead to the formation of radical derivatives or transitional components from lipid oxidation, a process triggered and accelerated by ACP treatment [18].

Similar results were observed in pearl millet samples treated with ACP in several studies [18, 34, 35] as well as in wheat samples [37]. However, these findings contradict those of some other studies. For example, Bahrami et al. [13] reported that in wheat flour treated with ACP (air, 15 V and 20 V for 60 and 120 s), the concentrations of total

nonstarch lipids, nonpolar lipids, and glycolipids did not change compared to the control. However, ACP treatment reduced the levels of free fatty acids and phospholipids in the samples treated at higher voltages. Similarly, Wu et al. [38] found no significant effect on the total fat content in bean flour treated with ACP. In a study by Thirumdas, Deshmukh, and Annappure [39], no significant difference in fat content was observed in basmati rice flour after ACP treatment. They noted a slight decrease in fat content in rice samples compared to the control, with no significant difference in other plasma-treated samples.

Similar to the present study, the fat content in Sarkar et al. [35] also increased with the duration of plasma treatment. Additionally, the findings of Bahrami et al. [13] were consistent with our results, showing that changes in fat content were dose-dependent. Furthermore, the observed decline in fat content with increasing voltage is likely due to the formation of complex compounds, where fats interact with other substances [18].

3.1.2. Ash Content. As shown in Table 1, the lowest and highest ash contents were found in the control and S2 samples, with values of 4.12% and 4.41%, respectively. Overall, plasma treatment slightly increased the ash content of chia flour samples. According to the table, the highest ash content in ACP-treated samples was observed in S2, with no significant difference compared to S3 ($p \geq 0.05$). Although there was an increasing trend in ash content with longer exposure times of ACP treatment at 6.5 kV, a decrease was observed in samples exposed to 8.5 kV. No significant difference was found between the control, S1, and S4 samples ($p \geq 0.05$). ACP treatment is known to break down organic matter in food, leading to an increase in the concentration of minerals. As organic components degrade, the relative proportion of minerals (which are less affected by ACP treatment) becomes higher. Additionally, reactive species generated by ACP treatment can interact with the surface of the food, potentially leading to the formation of new mineral compounds or the release of bound minerals [40]. These findings are consistent with those of Thirumdas, Deshmukh, and Annappure [39] and Burducea et al. [41], who reported an increase in ash content in basmati rice flour and wheat treated with ACP, respectively. However, Noppakun et al. [36] and Charu et al. [34] found no significant changes in the ash content of ACP-treated rice and millet samples, respectively.

3.1.3. Protein Content. As shown in Table 1, the protein content of the control sample was 23.98%, with the protein content of ACP-treated samples ranging from 23.65% to 24.85%. The lowest protein content was observed in S1, while the highest was in S2 (with no significant difference from S4). In other words, while ACP treatment initially caused a slight decrease in protein content, a subsequent increase was observed as exposure time increased at each voltage. ACP treatment influences the protein content of seeds through its derived reactive species, either by degradation or aggregation [35]. The susceptibility of protein to ACP treatment depends on factors such as its structure, grain compactness, and other constituents [36, 41–43].

It has been reported that specific radicals, such as hydroxyl and superoxide anions, generated during ACP treatment, have the potential to alter the molecular characteristics of proteins, leading to the denaturation of their structures [36]. Similar to the present study, Saberi et al. [44] observed a 49% increase in protein content in treated samples compared with the control, although they reported no significant change in protein content among different ACP-treated wheat samples, possibly due to variations in treatment time and power (80 W, 1–4 min). Studies by Thirumdas, Deshmukh, and Annapure [39] on basmati rice flour and Sarkar et al. [35] on pearl millet flour also supported the present findings. This increase in protein content could be attributed to the direct effect of various active radicals on the protein surface, resulting in modifications to protein structures or the conversion of proteins into peptides [35, 45].

In contrast, Kheto et al. [46] and Lokeswari et al. [18] found that the protein content of guar seed flour and pearl millet significantly decreased after ACP treatment, likely due to the use of longer exposure times and higher plasma power compared to the conditions in this study. Bahrami et al. [13], who studied the effect of ACP treatment on protein content in wheat flour, found no significant changes, with higher molecular weight proteins being generated due to protein oxidation triggered by plasma radicals. This finding was similar to the results reported by Noppakun et al. [36], Burducea et al. [41], and Wu et al. [38].

Yang et al. [47] observed that after ACP treatment of Chinese yam flour, the content of high molecular weight proteins (100–135 kDa) significantly decreased, while low molecular weight proteins (approximately 11 kDa) were notably increased. Therefore, it can be concluded that the ACP technique may degrade proteins into smaller molecular subunits, such as peptides and amino acids, and also alter their amino acid composition.

3.1.4. Crude Fiber Content. As shown in Table 1, the fiber content in the control sample was 29.80%, which significantly increased in the ACP-treated S1 and S3 samples, with values of 36.06% and 37.30%, respectively. Among the ACP-treated samples, S2 exhibited the lowest fiber content. The crude fiber content followed the trend: S3 > S1 > S4 > S2. In other words, although ACP treatment generally increased fiber content, a subsequent decrease was observed with prolonged exposure time, particularly at lower voltage levels. Interestingly, the crude fiber content in S2 was lower than

in the untreated sample, aligning well with previous studies [18, 36, 41]. However, the fiber content in the S1 and S3 samples was higher than the control, consistent with findings from Wu et al. [38].

The changes in crude fiber content in grains following ACP treatment can be attributed to the breakdown or modification of dense fibrous compounds into their subunits or byproducts due to reactive plasma radicals [18]. A negative correlation between the crude fiber and carbohydrate content ($r = -0.819$, $p < 0.05$) also exists. Crude fiber, primarily located in the cell wall, contributes to the bran coating. Therefore, it can be inferred that the plasma-induced corrosion of the bran may alter fiber content [36]. Additionally, the conversion of insoluble fibers into soluble fibers during plasma treatment could modify the crude fiber content in ACP-treated samples [38, 47], potentially making the fiber more accessible and measurable.

3.1.5. Carbohydrate Content. As shown in Table 1, the highest carbohydrate content was observed in the control sample (11.9%), highlighting the significant reducing effect of ACP treatment ($p < 0.05$). Among the treated samples, the lowest carbohydrate content was recorded in the S3 sample (3.5%). The carbohydrate content of ACP-treated samples followed the order S4 > S2 > S1 > S3. These findings indicate that the carbohydrate content in ACP-treated samples was influenced by both the voltage and the exposure time of the plasma treatment. Specifically, while an overall decreasing trend was observed with increasing ACP voltage, an increasing trend was noted with extended exposure time. This effect was more pronounced at higher voltages, where carbohydrate content increased by 192.85% compared to a 94.11% enhancement at lower voltages. The observed variations in carbohydrate content are likely attributable to the reactive species generated during ACP treatment, the type of plasma-generating gas, the specific treatment conditions (e.g., time and voltage), and the properties of the treated material (e.g., compactness and composition). The decreasing and increasing trends identified in this study may result from the depolymerization or condensation of carbohydrates facilitated by reactive species. Depolymerization likely involves the cleavage of glycosidic bonds, while oxidation of functional groups (particularly by reactive species like ozone) contributes to the reduction in carbohydrate content. These findings align with the studies of Kheto et al. [46] and Sarkar et al. [35], which reported decreased carbohydrate content in guar seed and pearl millet flour, respectively, following plasma treatment. The reasons for this decrease can be attributed to the depolymerization of carbohydrates (cleavage of glycoside bonds) and the oxidation of their functional groups by ACP-reactive species (especially ozone) [35, 48]. Conversely, Noppakun et al. [36] observed a significant increase in carbohydrate content in treated rice samples compared to controls. Similarly, Thirumdas, Deshmukh, and Annapure [39] and Wu et al. [38] reported no significant changes in the carbohydrate content of basmati rice and bean flours, respectively, after ACP treatment.

3.1.6. In Vitro Starch Digestibility. Starch is a crucial component of flour used in bakery products [49], and its digestibility

TABLE 2: Effect of plasma treatment on digestibility of chia flour.

Parameters	Treatments				
	Control	S1	S2	S3	S4
Starch digestibility (%)	40.14 ± 0.79 ^d	41.59 ± 0.98 ^d	56.20 ± 0.68 ^c	61.90 ± 0.14 ^b	64.99 ± 0.49 ^a
Protein digestibility (%)	68.39 ± 0.40 ^c	68.95 ± 0.76 ^c	79.26 ± 0.51 ^b	83.43 ± 0.54 ^{ab}	85.54 ± 0.38 ^a

Note: Data are reported as *average ± standard deviation*. Values followed by different letters in each row are significantly different ($p < 0.05$). Control is an untreated sample, and S1, S2, S3, and S4 are plasma-treated flours for 3 min at 6.5 kV, 8 min at 6.5 kV, 3 min at 8.5 kV, and 8 min at 8.5 kV, respectively.

significantly contributes to the nutritional value of these products [50]. Numerous studies have shown that starch digestibility is primarily influenced by its structure, particularly the amylose content. However, nonstarch components such as proteins and lipids, their interactions with starch, the presence of antinutrients or enzyme inhibitors, and processing conditions are also critical factors affecting starch digestibility [42, 51]. Table 2 presents the *in vitro* starch digestibility of ACP-treated chia flour samples compared to the control. As indicated, ACP treatment markedly enhances the *in vitro* starch digestibility of chia flour, with untreated samples showing 40.14% digestibility. A significant increasing trend is observed with escalating ACP treatment intensity, culminating in the highest digestibility (64.99%) for the S4 treatment (8 min at 8.5 kV) ($p < 0.05$). These results align with those of prior studies [41, 52–55], which suggest that ACP treatment alters starch's amylose and amylopectin content and their ratio [56]. The observed increase in starch digestibility is likely due to depolymerization and degradation of starch molecules caused by reactive species generated during ACP treatment. These modifications enhance the hydrolysis rate of starch molecules, making them more accessible to digestive enzymes [54]. Additionally, Wu et al. [38] hypothesized that this improvement in digestibility might be attributed to the inactivation of α -amylase inhibitors in starch by ACP treatment. Their findings also confirmed a positive correlation between digestibility enhancement and treatment time and power, consistent with the results of the present study. In conclusion, ACP treatment offers an effective alternative to conventional chemical and physical methods for modifying native starch. It overcomes the weak physicochemical and functional properties of native starch, making it more suitable for application in food products [52].

3.1.7. *In Vitro* Protein Digestibility. Proteins are essential macronutrients in the human diet, serving as critical building blocks for growth and maintenance. To be utilized effectively, dietary proteins must be hydrolyzed into their basic components, namely, amino acids or small peptides, which are digestible by consumers. This hydrolysis is facilitated by various protein-digesting enzymes or peptidases in the human digestive system [57]. Protein digestibility is influenced by both intrinsic and extrinsic factors. Intrinsic factors include the amino acid profile, protein folding, and the degree of crosslinking within the protein structure. Extrinsic factors, on the other hand, encompass environmental conditions such as pH, temperature, and ionic strength, as well as the presence of molecules like emulsifiers and antinutritional agents [57]. Given these variables, differences in protein

digestibility among various food sources can often be attributed to variations in protein structure, the nature of amino acid linkages, and their bioavailability. These factors collectively determine the accessibility and efficiency of protein hydrolysis, making digestibility an important consideration in assessing the nutritional quality of protein sources [11].

As presented in Table 2, the lowest *in vitro* protein digestibility was observed in the control sample (68.39%), while the highest digestibility was noted in the S4 sample (85.54%). These findings demonstrate that ACP treatment significantly improves protein digestibility in chia flour ($p < 0.05$). Furthermore, an increasing trend in protein digestibility was observed with longer exposure times and higher voltages during ACP treatment.

The results align with those of previous studies, such as Kheto et al. [46], though research on the effects of ACP treatment on protein digestibility and protease activity remains limited. This underscores the need for further investigations in this area. Protein digestibility is a critical indicator of protein quality, reflecting the accessibility and breakdown of protein into absorbable forms. The observed enhancement in digestibility is likely due to the oxidation process triggered by active species generated during ACP treatment. A strong correlation existed between protein digestibility and starch digestibility parameters ($r = 0.998$, $p < 0.05$). These reactive species can modify the side chains of amino acids and disrupt protein molecules, leading to structural unfolding and degradation. The increased digestibility can be attributed to the opening of the protein structure, which facilitates the accessibility of protease enzymes. Additionally, ACP treatment may neutralize or eliminate natural inhibitors of protease activity, further promoting the enzymatic breakdown of proteins [11].

3.2. Polyphenol Content. Polyphenols, which include compounds such as flavanols, hydroxycinnamic acids, anthocyanins, and lignans, are important dietary components. These secondary metabolites produced by plants have garnered significant interest in the food industry due to their health-protective properties. Polyphenols are broadly classified into flavonoid and nonflavonoid compounds, both of which exhibit high antioxidant activity. Thanks to their protective effects, polyphenols contribute to numerous health benefits, including antioxidant, anti-inflammatory, and antimicrobial activities. These properties make them valuable in the prevention of various diseases, such as cardiovascular diseases, cancer, diabetes, and obesity [58]. It is important to note that phenolic compounds can exist in plants in two forms: free or bound to other molecules [59].

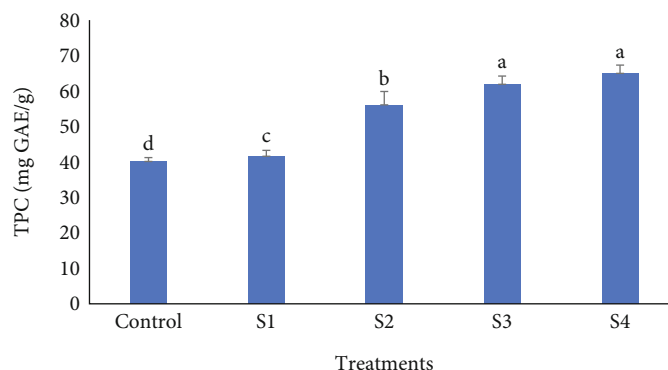


FIGURE 1: Effect of plasma treatment on polyphenol content of chia flour. Control is an untreated sample, and S1, S2, S3, and S4 are plasma-treated flours for 3 min at 6.5 kV, 8 min at 6.5 kV, 3 min at 8.5 kV, and 8 min at 8.5 kV, respectively. Different lowercase letters indicate a significant difference ($p < 0.05$) between samples.

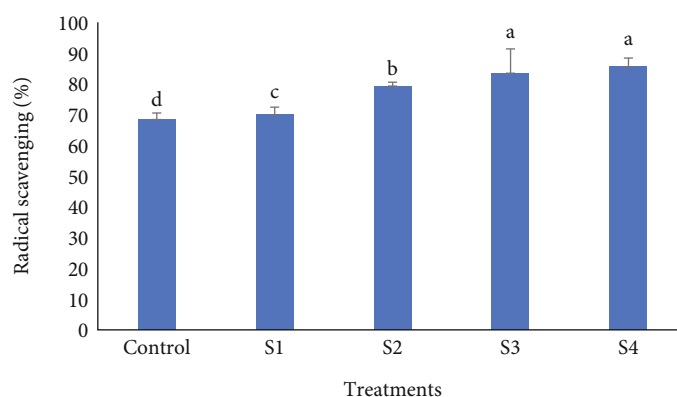


FIGURE 2: Effect of plasma treatment on antioxidant activity of chia flour (by DPPH assay). Control is an untreated sample, and S1, S2, S3, and S4 are plasma-treated flours for 3 min at 6.5 kV, 8 min at 6.5 kV, 3 min at 8.5 kV, and 8 min at 8.5 kV, respectively. Different lowercase letters indicate a significant difference ($p < 0.05$) between samples.

Figure 1 illustrates the impact of plasma treatment on the polyphenol content of chia flour compared to the control. The lowest polyphenol content was observed in the control sample, with a value of 40.14 mg gallic acid equivalent (GAE)/g. Plasma treatment resulted in a noticeable increase in total polyphenol content in all treated samples compared to the control. The results indicate that the polyphenol content was influenced by both the voltage and treatment time. Among the treated samples, S1, which underwent the shortest exposure time and the lowest voltage, exhibited the lowest polyphenol content (41.59 mg GAE/g). Conversely, the S4 sample, treated under the highest voltage and the longest time, showed the highest polyphenol content at 64.98 mg GAE/g.

Similar findings have been reported in numerous previous studies [21, 39, 41, 44, 46, 60–62], supporting the observed increase in polyphenol content. This increase is likely attributed to the breakdown of covalent bonds between phenolic compounds and polysaccharides in the cell wall layers or glycosidic components. Additionally, the fragmentation of larger phenolic compounds into smaller, more measurable components, facilitated by plasma-induced reactive ions and radicals, has been proposed by other researchers [39, 56, 63]. However, contrary findings have been reported in some studies, which observed a decrease in phenolic content due to the degrada-

tion of phenolic compounds by reactive oxygen species (ROS) at higher treatment voltages and prolonged exposure times [34, 35, 64–66].

3.3. Antioxidant Capacity. Antioxidants are molecules that inhibit the oxidation of other substances. The antioxidant capacity of foods is determined by the presence of compounds capable of delaying or inhibiting oxidation within the food matrix. Antioxidants play a crucial role in extending the shelf life of food products by neutralizing free radicals and delaying lipid peroxidation, a major contributor to food spoilage. Additionally, they provide protective effects for the human body against the harmful impacts of free radicals. Vitamin C, tocopherols, carotenoids, and flavonoids are among the most well-known antioxidant compounds in the human diet [67]. The DPPH method is one of the most commonly used techniques for evaluating antioxidant activity. This method measures the capacity of compounds to donate electrons and neutralize free radicals [68].

Figure 2 illustrates the effect of plasma treatment on the antioxidant activity (percentage of DPPH inhibition) of chia flour compared to the control sample. The lowest antioxidant activity was observed in the control sample (68.39%), whereas the highest activity was noted in the S4 sample

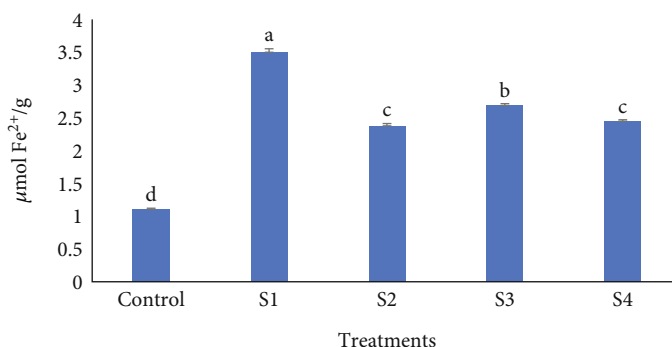


FIGURE 3: Effect of plasma treatment on antioxidant activity of chia flour (by FRAP assay). Control is an untreated sample, and S1, S2, S3, and S4 are plasma-treated flours for 3 min at 6.5 kV, 8 min at 6.5 kV, 3 min at 8.5 kV, and 8 min at 8.5 kV, respectively. Different lowercase letters indicate a significant difference ($p < 0.05$) between samples.

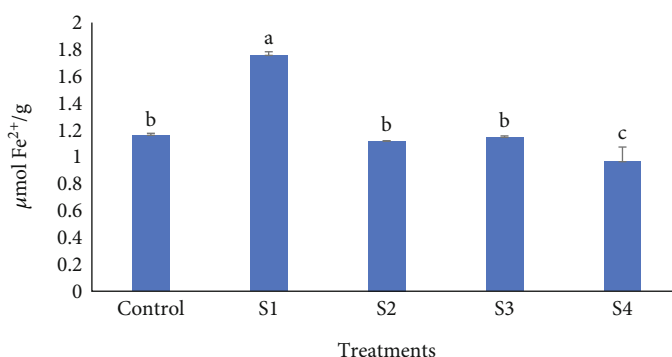


FIGURE 4: Effect of plasma treatment on antioxidant activity of chia flour (by reducing power assay). Control is an untreated sample, and S1, S2, S3, and S4 are plasma-treated flours for 3 min at 6.5 kV, 8 min at 6.5 kV, 3 min at 8.5 kV, and 8 min at 8.5 kV, respectively. Different lowercase letters indicate a significant difference ($p < 0.05$) between samples.

(85.451%). The results clearly demonstrate that plasma treatment significantly enhanced the antioxidant activity of chia flour samples ($p < 0.05$). Similar findings were reported by Sarangapani et al. [56], who investigated the effects of ACP treatment on rice flour. The observed increase in antioxidant activity following plasma treatment can be attributed to the action of persistent plasma species, which facilitate the release of phenolic compounds. Well correlation existed between polyphenol content and DPPH values of chia flour through ACP treatment ($r = 0.896$, $p < 0.05$). Furthermore, the presence of UV radiation during plasma production likely contributes to the formation of secondary metabolites, such as polyphenols and flavonoids, which are known to enhance antioxidant properties [39].

Generally, it appears that the antioxidant activity values were influenced by both voltage and treatment time, with the trend of change mirroring that of the total polyphenol content. Among the treated samples, the S1 sample, exposed to the lowest voltage and treatment time, exhibited the lowest antioxidant activity at 41.59%. In contrast, the S4 sample, treated with the highest voltage and treatment time, showed the highest antioxidant activity at 85.541%.

The FRAP assay is another widely used method for evaluating antioxidant activity, which is based on measuring the reduction of ferric ions (Fe^{3+}) and electron transfer in an acidic medium [68]. Reducing power is another common method to assess antioxidant activity, as reducing agents

can inhibit lipid peroxidation. Generally, the antioxidant capacity measured by the reducing power assay is directly related to the presence of reducing compounds [69].

Figures 3 and 4 illustrate the effect of plasma treatment on the antioxidant activity of chia flour, as measured by the FRAP and reducing power assays, respectively. As shown in these figures, the lowest antioxidant activity was observed in the control sample and S4 treatment, with values of 1.10 and 0.96 $\mu\text{mol Fe}^{2+}/\text{g}$ ($p < 0.05$), respectively. On the other hand, the highest antioxidant activity for both assays was detected in the S1 sample (with the lowest voltage and treatment time), with values of 3.5 and 1.76 $\mu\text{mol Fe}^{2+}/\text{g}$, respectively. For the FRAP method, plasma treatment significantly increased the antioxidant activity of chia flour samples ($p < 0.05$). However, in the reducing power method, antioxidant activity in all treatments (except S1) was lower than in the control sample. The results from both methods suggest that plasma treatment with shorter time and lower power (as in S1, with 3 min and a voltage of 6.5 kV) increased antioxidant activity. In contrast, increasing treatment time and voltage resulted in a decrease in antioxidant activity, with a significant reduction observed in most cases (except for S4 being higher than S2 in the FRAP method with 8-min treatment time).

The antioxidant activity measured by the DPPH method showed fewer changes, which may indicate its lower sensitivity compared to other methods. The FRAP and reducing

TABLE 3: Pearson's correlation analysis of variables of the present study.

Parameter	Pearson's correlation										
	Fat	Ash	Protein	Fiber	Carbohydrate	Polyphenol	DPPH	SD	PD	FRAP	Reducing power
Fat	1										
Ash	0.279	1									
Protein	0.234	0.502	1								
Fiber	-0.579	0.275	0.187	1							
Carbohydrate	0.495	-0.371	0.206	-0.819	1						
Polyphenol	0.639	0.495	0.174	-0.126	-0.143	1					
DPPH	0.539	0.437	0.198	-0.007	-0.204	0.896	1				
Starch digestibility (SD)	0.646	0.537	0.233	-0.11	-0.138	0.982	0.947	1			
Protein digestibility (PD)	0.658	0.52	0.204	-0.125	-0.132	0.978	0.946	0.998	1		
FRAP	-0.969	-0.178	-0.23	0.533	-0.497	-0.652	-0.588	-0.664	-0.674	1	
Reducing power	-0.582	0.323	-0.059	0.523	-0.756	0.179	0.207	0.18	0.162	0.596	1

Note: Strongly correlation (0.8–1), highly correlation (0.6–0.8), medium correlation (0.4–0.6), low correlation (0.2–0.4), and no correlation (0.0–0.2).

power methods generally followed a similar trend, with an initial increase in antioxidant activity followed by a decline as ACP treatment time and voltage increased. This trend was also observed in the study by Tappi et al. [63]. The observed increase in antioxidant activity could be attributed to the higher levels of total polyphenols, suggesting a strong correlation between the two ($r = 0.896$, $p < 0.05$). Additionally, it has been reported that UV radiation produced during ACP treatment can stimulate the synthesis of secondary metabolites, especially polyphenols and flavonoids [39]. Furthermore, free radicals generated during ACP treatment may act as stress signals, triggering stress reactions that lead to increased antioxidant synthesis [56]. The decrease in antioxidant activity in the FRAP and reducing power assays with prolonged treatment time and higher voltage may be due to the reaction of free radicals with antioxidant compounds, forming intermediate compounds, or the degradation of phenolic compounds [56]. Similar results have been reported in previous studies, such as the effect of ACP on antioxidant activity in brown rice grains (measured by DPPH and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) methods) [61], basmati rice flour (by reducing power) [39], parboiled rice flour (by reducing power) [56], and apple slices (using DPPH, FRAP, etc.) [63]. However, some studies have reported no effect of plasma treatment on antioxidant activity, such as those on fresh-cut kiwifruit, wheat, and foxtail millet [41, 65, 70].

On the other hand, Charu et al. [34] reported a decrease in antioxidant activity in pearl millet treated by ACP, which they attributed to the degradation of natural antioxidants caused by the high-energy reactive species produced during plasma treatment. This also led to a reduction in the performance of antioxidants. Similarly, Li et al. [64] also disagreed with the findings of the present study. In this regard, it should be noted that the observed differences in the effects of ACP on various factors, such as nutritional and antioxidant properties, across different studies can be attributed to several factors. These

include variations in the applied power (voltages), treatment time, and gas used, as well as differences in sample types and the treatment environment [36, 71].

3.4. Correlation Analysis. This analysis was done to investigate the strength and direction of the correlation between the investigated parameters. As demonstrated in Table 3, a strong negative correlation existed between fiber and carbohydrate content ($r = -0.819$, $p < 0.05$). In other words, as hypothesized through the text, quantitative changes observed in grains of crude fiber through ACP treatment are attributed to the breakdown and/or modification of dense fibrous carbohydrates into their subunits or byproducts [18]. Considering antioxidant capacity, a positive strong correlation existed between polyphenol and DPPH parameters ($r = 0.896$, $p < 0.05$). Secondary metabolite formation through ACP treatment is considered the main reason for the enhancement observed at antioxidant capacity [39]. As demonstrated in Table 3, a strong correlation existed between in vitro starch digestibility and protein digestibility and the DPPH assay (with exact r values of 0.947 and 0.946, respectively, $p < 0.05$). Also, a stronger correlation existed between polyphenol and in vitro starch digestibility and protein digestibility (with r values equal to 0.982 and 0.978, respectively, $p < 0.05$). In other words, cleavage induced by reactive species enhanced the protein/starch digestibility along with more release of phenolic compounds.

Pearson's correlation assessment declared a negative correlation ($r = -0.969$, $p < 0.05$) between the fat content and FRAP analysis (as declared in Table 3). In other words, a decline in total antioxidant activity as measured by the FRAP assay by increasing the intensity of ACP treatment could be explained by an enhancement in fat content. As the FRAP assay also investigates the bioavailability of antioxidants through processing [72], it seems that increasing the intensity of ACP treatment decreased the bioavailability of antioxidants by increasing the fat content and its susceptibility to being oxidized by hydroxyl and superoxide anions [36].

4. Conclusion

This study investigates the impact of ACP treatment on the nutritional characteristics of chia seeds, focusing on their proximate composition, phenolic content, and antioxidant activities. As demonstrated, the plasma-activated species induced by ACP treatment potentially led to depolymerization, structural modifications, and conversions of chia seed constituents, depending on the treatment intensity. The intensity of ACP treatment was evaluated in terms of voltage and exposure time, and the observed differences in the effects of these two parameters may be attributed to their influence on the concentration of activated species.

Data Availability Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

All authors contributed to the study concept and design. Amene Nematollahi: supervision, writing original draft, project administration, review and editing; Narjes Jamali, Zahra Rahmdar, Mohammad Kiani, and Mohammad Torkashvand: formal analysis, data curation, writing some parts of the original draft; Neda Mollakhalili-meybodi: project administration, methodology, review and editing. All authors read and approved the final manuscript.

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