Allergol Immunopathol (Madr). 2023;51(SP1):1–13 eISSN:1578-1267, pISSN:0301-0546

Allergologia et immunopathologia
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica
www.all-imm.com

ORIGINAL ARTICLE

Evaluating the influence of cold plasma bubbling on protein structure and allergenicity in sesame milk

Dharini Manoharan, Anbarasan Rajan, Jaspin Stephen, Mahendran Radhakrishnan*

Centre of Excellence in Non-Thermal Processing, National Institute of Food Technology, Entrepreneurship, and Management, Thanjavur (NIFTEM-T, formerly Indian Institute of Food Processing and Technology), Ministry of Food Processing Industries (MoFPI), Government of India, Pudukkottai Road, Thanjavur, Tamil Nadu, India

Received 5 October 2022; Accepted 21 December 2022
Available online 13 March 2023

KEYWORDS
allergenicity;
circular dichroism;
EPR;
plasma bubbling;
sesame milk

Abstract
Background: Sesame is a traditional oilseed comprising essential amino acids. However, the presence of allergens in sesame is a significant problem in its consumption; thus, this study attempted to reduce these allergens in sesame oilseeds. *Objective:* The present study aimed to evaluate the effect of cold plasma processing on structural changes in proteins, and thereby the alteration of allergenicity in sesame milk. *Method:* Sesame milk (300 mL) was processed using atmospheric pressure plasma bubbling unit (dielectric barrier discharge, power: 200 V, and airflow rate: 16.6 mL/min) at different exposure times (10, 20, and 30 min). *Results:* The efficiency of plasma-bubbling unit as measured by electron paramagnetic resonance in terms of producing reactive hydroxyl (OH) radicals proved that generation of reactive species increased with exposure time. Further, the plasma-processed sesame milk subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and differential scanning calorimetery analysis revealed that plasma bubbling increased the oxidation of proteins with respect to bubbling time. The structural analysis by Fourier transform infrared spectroscopy and circular dichroism revealed that the secondary structure of proteins was altered after plasma application. This change in the protein structure helped in changing the immunoglobulin E (IgE)-binding epitopes of the protein, which in turn reduced the allergen-binding capacity by 23% at 20-min plasma bubbling as determined by the sandwich-type enzyme-linked immunosorbent assay. However, 30-min plasma bubbling intended to increase allergenicity, possibly because of increase in IgE binding due to the generation of neo epitopes.
Conclusion: These changes proved that plasma bubbling is a promising technology in oxidizing protein structure, and thereby reducing the allergenicity of sesame milk. However, increase in binding at 30-min bubbling is to be studied to facilitate further reduction of the binding capacity of IgE antibodies.

***Corresponding author**: Mahendran Radhakrishnan, Associate Professor and Head, Centre of Excellence in Non-Thermal Processing, National Institute of Food Technology, Entrepreneurship, and Management Thanjavur (NIFTEM-T), Ministry of Food Processing Industries (MoFPI), Government of India, Pudukkottai Road, Thanjavur 613005, Tamil Nadu, India. *Email address*: mahendran@iifpt.edu.in

https://doi.org/10.15586/aei.v51iSP1.783

Introduction

Mammillary milk is a rich source of protein and is consumed globally, irrespective of age, gender, and location. However, owing to the cost of production, maintenance, and impact of the environment on cattle rearing, people have started to shift from animal to plant-based products.[1] Furthermore, the reduced availability of animal protein in developing countries and the trend to change from animal protein to plant-based protein in developed countries has given us an idea of discovering new plant-based milk.[2] The primary plant materials that are known for producing plant-based milk are soy, almond, oat, peanut, hazelnut, rice, lupin, millet, and oats.[3] In the past, more importance was given to soybean, cashew, almond, and peanut; however, now it has been shifted to low-cost, highly nutritious plant sources, such as legumes and oil seeds. Recently, light has been shed on sesame; it is considered a potential alternative of animal protein that can be incorporated into daily food diet to increase protein content. It is reported to be the cheapest source of protein in addition to the profound health-beneficial nature of seeds.[4] It has been reported that of the individuals who are sensitive to peanuts, 25–40% are also sensitive to sesame seeds.[5] Sesame, a valuable traditional oilseed crop, is known as the queen of oilseeds because of its unique aroma, taste, and more oxidative stability of sesame lipids.[6] Sesame seeds are often processed into sesame oil, which is rich in antioxidants, such as polyunsaturated fatty acids, lignans, tocopherols, phytosterols, bioactive compounds, and antioxidants that are highly beneficial for health.[2] Sesame consists of essential proteins, compared to meat and cereals; it also contains all essential amino acids, compared to other plant-based proteins.[7] However, the potential plant-based source (sesame) poses a significant threat of causing allergies in sensitized individuals.[8,9] In the last decade, the world has seen an enormous increase in sesame-related allergies in individuals.[10] Therefore, in 2021, the Food and Drug Administration (FDA) added sesame as the ninth food allergen in the list of allergy-causing food items.[11] Allergic reactions can be caused by proteins, such as lipid transfer proteins and seed storage proteins.[12] The primary seed storage proteins present in sesame are potent enough to cause sesame allergy; it includes 11S albumin, 2S albumin, 7S globulin, and oleosins.[13]

The intake of unprocessed sesame poses a significant threat in terms of indigestion of seeds and presence of allergens.[14] Therefore, sesame needs to be processed before supplying it as an alternative to animal milk. Many processing methods have been used to reduce allergenicity in plant-based foods;[15] however, processing plant-based foods with thermal treatments, such as roasting and hot-water treatments, could affect the quality of plant foods.[16] Therefore, non-thermal processing technologies would be beneficial in retaining whole nutrients found in these plant-based foods. Following this, many non-thermal processing methods are gone through to reduce indigestibility and activity of allergens.[2,17–19]

Cold plasma is a non-thermal processing technology that has been reported to cause changes in protein structure and alter the allergenicity of food material.[20] Different reactive species present in plasma aid in altering the structure of protein (modify the epitopes) by fragmentation or aggregation, which in turn helps in changing the percentage of its binding with immunoglobulin E (IgE) antibody.[10] To the best of our knowledge, cold plasma processing technology on sesame seeds or sesame milk has not been studied to reduce its allergenicity. Thus, the present study is aimed to study cold plasma's effect in changing the structure and allergenicity of sesame milk protein. The plasma processing was evaluated using Electron Paramagnetic Resonance (EPR) spectroscopy, and the changes in sesame milk were evaluated with the aid of differential scanning calorimeter (DSC), Fourier Transform Infrared (FTIR) spectroscopy, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), circular dichroism (CD) spectroscopy, and sandwich-type Enzyme-Linked Immunosorbent Assay (ELISA).

Materials and Methods

Raw material preparation

Raw sesame (variety: *swetha til*) was procured from the Indian Institute of Oil Research, Hyderabad, India. First, sesame seeds were cleaned, and a known quantity of sesame was soaked overnight in distilled water. Next, the soaked seeds were ground in the tabletop mixer grinder; six parts of distilled water were added to one part of grounded sesame seeds. Then, using a two-layer muslin cloth, the grounded mixture was filtered manually (twice). The resulting plant extract/milk was used for experiments.

Experimental setup and design

The system used for this study was a plasma bubbling unit, as represented in Figure 1. The system comprised a simple dielectric barrier discharge (DBD) chamber for generating plasma, a blower for supplying air inside the DBD unit, a transformer for supplying current to the DBD unit, two variacs for supplying electric current to the transformer, and a blower for providing air to DBD unit. In addition, it contained a tube for supplying ionized air from DBD unit to the target. The DBD unit comprised two hollow concentric cylinders (electrodes)—one made of solid aluminium (6.5 [length] × 2.1 cm [diameter]) and the other made of meshed aluminium sheet (5.5 [length] × 1.1 cm [diameter]). The meshed aluminium sheet was cased inside a quartz tube (6 [length] × 1.8 [diameter] × 0.1 cm [thickness]) and was connected to the ground. Two aluminium cylinders, along with a quartz tube, were connected using two end Teflon attachment rings. The solid aluminium cylinder (connected to a power supply) and the meshed aluminium concentric cylinder enclosed in a quartz tube were mounted with Teflon rings so that 0.3-mm space was left between both electrodes. When power was applied, the space between quartz and solid outer cylinder was ionized (plasma was generated). Gas/air was supplied to this space for carrying the ionized gas produced within the DBD unit. Provisions were provided on both end attachment rings for the inlet and outlet of air. Two separate variacs were used

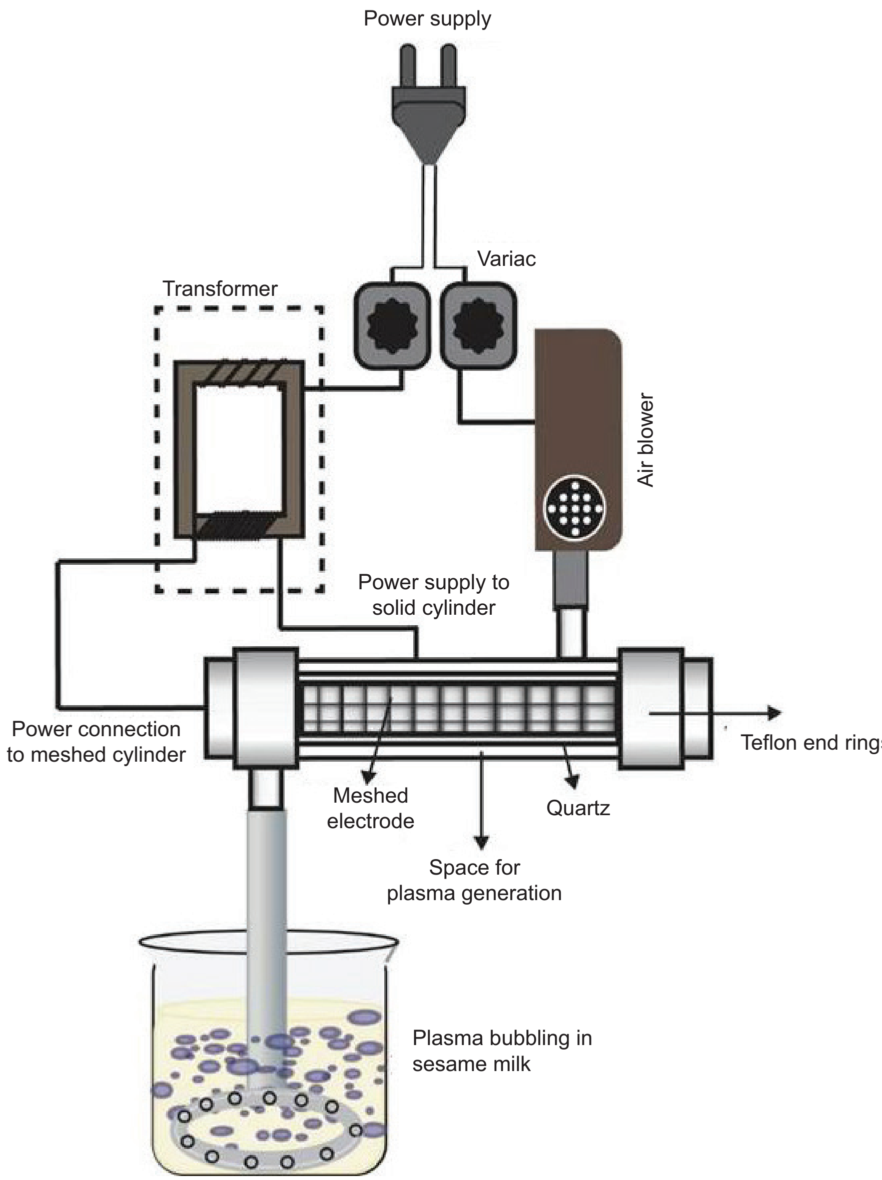

Figure 1 Schematic representation of plasma-bubbling unit.

for supplying power to the transformer and the air blower. The air ionized inside the DBD was continuously removed (air blower) and supplied to the sesame milk in the form of ionized air bubbles.

Sesame milk (300 mL) was treated inside a food-grade aluminium can. In order to maintain uniformity of ionized air bubble supply, the tube from the plasma unit was connected to a circular perforated tube; this aided in the constant supply of ionized gas into the sesame milk. Based on the previous studies on the plasma bubbling unit, the power level of 200 V and the air supply of 16.6 mL/min were used in the present study.[21] In addition, the exposure time (10, 20, and 30 min) of plasma was altered to identify the effect of plasma exposure time on protein structure and allergenicity.

Electron paramagnetic resonance spectroscopy

The hydroxyl (OH) radical present in the plasma-treated sesame milk was estimated using EPR spectroscopy. The EPR equipment and operating procedure used in this study were similar to the equipment used to study on Neera processing,[22] with the following dimensions—model: Bruker EMX Plus, microwave frequency: 9.800127 GHz, centre field: 3480, power supply: 10 mW, and number of scans: 40. Spin-trapping agents were used to trap free radicals to extend their lifetime. The common spin trap agent used for OH radical identification for ERP analysis was 5,5-dimethyl-1-pyrroline N-oxide (DMPO). The raw sesame milk + 50-mM DMPO mixture was subjected to plasma bubbling for 10, 20, and 30 min exposure time. The DMPO-OH formed after plasma bubbling was characterized using EPR spectroscopy. The DMPO-OH spectrum was a signal with four lines with a peak ground acceleration, g = 2.009.

Characterization of the effect of plasma on sesame milk

Thermal properties

The thermal properties of sesame milk were determined using DSC (3 Star system, Mettler Toledo, Hong Kong). A milk sample of 10 µL was used for analysis using continuous nitrogen gas flushing at a rate of 20 mL/min. The apparatus was calibrated using standard Indium. The milk samples were placed on an aluminium sample holder and heated from 0 to 300°C at a rate of 10°C/min.[23] The thermal behavior of milk samples, such as heating onset, peak temperature, and enthalpy, was calculated.

Protein profiling of sesame milk

The impact of cold plasma on protein profile was determined using the SDS-PAGE electrophoresis method.[24,25] The sesame milk samples were diluted with distilled water to obtain approximately 100–500 µg of protein in the sample. The diluted sample was mixed with sample loading buffer (bromophenol dye) in a sample–loading buffer ratio of 1:1.5. The mixture was then heated in a water bath at 90°C for 10 min and cooled down. The samples were then centrifuged at 1000 rpm for 15 min. The prepared samples and the pre-stained ladders were loaded onto the wells of stacking buffer. After loading, the equipment was run at a constant power level of 100 V and 200 mA. When power was applied, the samples loaded onto the stacking gel moved toward the separating gel. After electrophoresis, the gel was stained in Coomassie blue dye overnight and later destained using the destaining buffer. The destained gel was then documented to identify the presence or absence of protein bands in sesame milk.

Structural characterization of sesame milk protein

Fourier transform–infrared spectroscopy

Structural changes in control and plasma-treated sesame milk samples were analyzed by subjecting freeze-dried milk samples to FTIR analysis in Shimadzu IRAffinity-1S spectrometer (Analytical and Measuring Instruments, Japan). As the equipment uses attenuated total reflectance (ATR), a freeze-dried sample was used for analysis. This method involves applying the sample above the ATR crystal and measuring the spectra from the infrared light that was internally reflected in the crystal. The spectroscopic scan was performed in the mid-infrared range of 5000–400 cm^{-1} with a resolution of 4 cm^{-1} in potassium bromide (KBr) beam splitter and 45 scans per measurement. The infrared (IR) solution program was used to identify spectra peaks.

Circular dichroism

Freeze-dried sesame milk was subjected to circular dichroism to analyze the secondary structure of unprocessed and plasma-processed sesame milk samples. The samples were prepped based on the method described in a freeze-dried almond milk powder study.[26] A 0.01-M phosphate buffer at a pH of 7 (sterilized) was used for dissolving the freeze-dried powder. The freeze-dried powder–phosphate buffer ratio of 1:20 was used for the analysis. In order to extract the essence required for analysis, the mixture was incubated for 12 h at 4°C. After incubation, the mixture was centrifuged for 10 min, and the supernatant was used for analysis. Further, the extract was diluted with the same phosphate buffer solution at the time of analysis so that the voltage used for analysis was below 700 V. Both control and plasma-treated samples were scanned at a range of 260–190 nm with 1-mm bandwidth and 0.2-nm pitch.

IgE-binding capacity of sesame milk antigen

Sandwich-type ELISA was performed using the sesame allergen ELISA kit (BioCheck, UK). As the kit was specific for sesame seeds, the microtiter plate provided was coated with sesame storage protein antibody. Thus, the diluted sesame milk sample (containing approximately 50-µg protein) was applied to the microtiter plate and incubated for 20 min.[27,28] After incubation, the microtiter wells were washed with the washing solution provided with the kit to remove excess unbound material. The given peroxidase-conjugated second antibody that acts against the sesame storage protein was applied to the wells, incubated for 20 min, and washed off. Later the substrate solution was added to the wells, generating a blue color. The color development was stopped after 20 min of incubation with a stop solution, which turned blue color into yellow. Then, the yellow color was measured using a spectrophotometer at 450 nm. The color intensity developed directly indicated the sesame seed protein allergenicity (IgE-mediated bonding percentage) in sesame milk. The results were expressed as percentage reduction in IgE binding, compared to the untreated sesame milk sample.

Data processing and statistical analysis

As most of the parameters characterized in this work are based on instrumental methods, replications were not performed, except for ELISA analysis. The OriginPro software was used for producing graphs from EPR, DSC, FTIR, and circular dichroism data. OriginPro was also utilized for analyzing the area under the peak obtained in FTIR. The CD pro (online) software was used for analyzing the circular dichroism data. The statistical analysis of sandwich-type ELISA was performed using the SPSS software (version 20.0) using ANOVA with Tukey's honestly significant difference (HSD) test, with a significance level set at $P < 0.05$.

Results

Electron paramagnetic resonance spectroscopy

The EPR spectroscopy was applied to determine the concentration of reactive species with respect to the plasma exposure time. It was observed that the intensity of the signal obtained from the plasma-treated sesame milk increased with increase in exposure time (Figure 2). The intensity of the highest peak with g-force = 2.008 was chosen to understand which treatment would form more

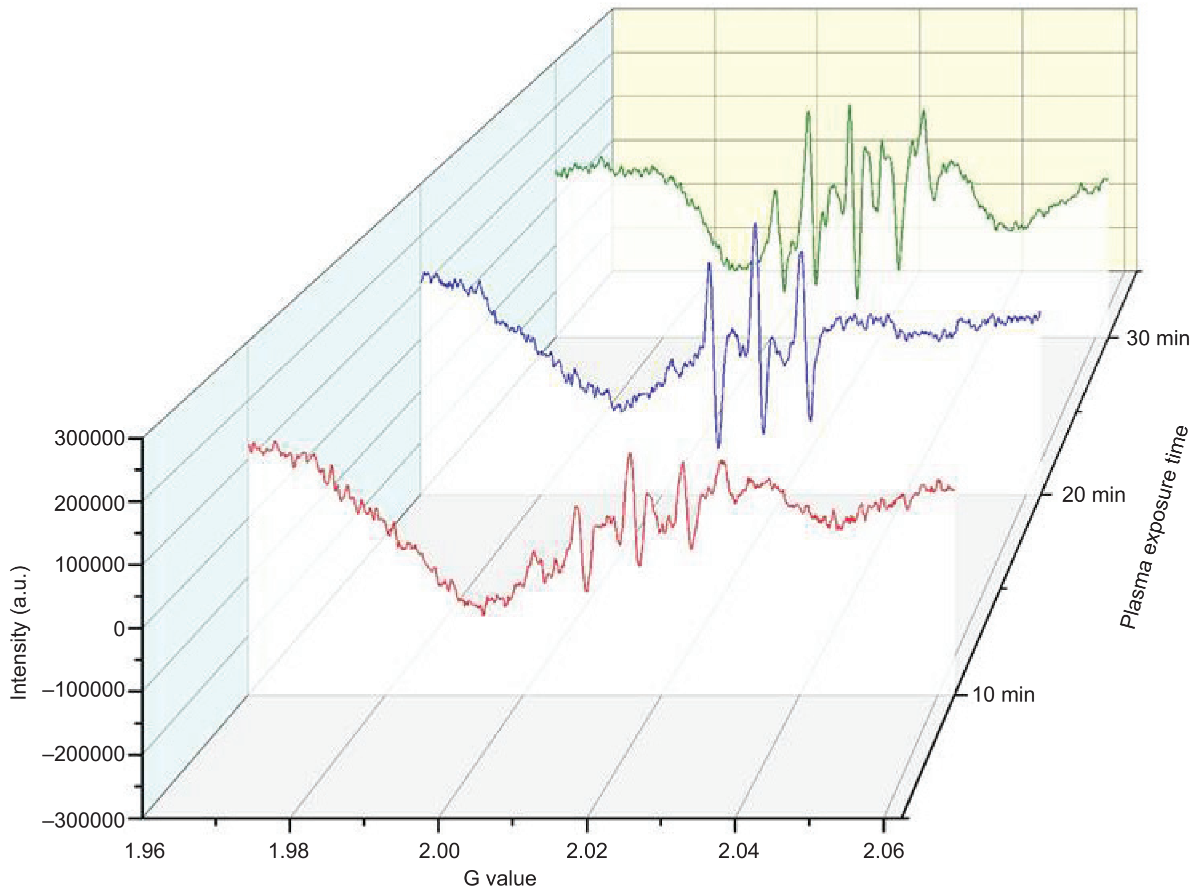

Figure 2 EPR spectra of plasma-treated sesame milk.

quantities of DMPO-OH adducts. It was observed that the 20-min bubbling intended to have more intensity than the 10- and 30-min plasma bubbling time. The intensity of the 10-min plasma-bubbled sample had less intensity, whereas the intensity of the 30-min plasma-bubbled sample was greater than 10 min but lesser than 20 min.

Thermal profile analysis (DSC)

In this study, the sesame milk samples were subjected to heat from 0°C to 300°C. Figure 3 presents the thermograms of control and plasma-treated sesame milk samples. Almost all thermograms revealed a single deep endothermic peak at an approximate denaturation or peak temperature (165–170°C approx.). The endothermic peaks obtained in the present study, the onset temperature, the peak or denaturation temperature, and the enthalpy of the control and the plasma-treated samples were presented in Table 1. The onset temperature of raw sesame milk was 165.95°C, which changed to 169.59°C, 165.83°C, and 166.02°C plasma-bubbled samples at 10-, 20-, and 30 min, respectively. The peak denaturation temperature also varied with respect to plasma treatment. However, the change does not follow any trend; the peak temperature in raw milk (169.65°C) increased to 173.07°C at 10 min whereas at 20 min (169.95°C), it remained similar to that of the control. At 30-min plasma-bubbled sample, the peak value was observed at 170.79°C. In this study, the enthalpy value decreased with increase in the processing condition. The enthalpy of raw sesame milk was 1360.03 J/g, which decreased in plasma-processed sesame milk samples. The enthalpy at 10-, 20- and 30-min plasma-bubbled samples decreased to 1330.13 J/g, 1251.41 J/g, and 1240.28 J/g, respectively.

Sesame protein profiling (SDS-PAGE)

The SDS-PAGE analysis (Figure 4) demonstrated the documentation of SDS gel for the marker, control, and three plasma-treated samples. Three distinct bands between 20 kDa and 25 kDa and two bands below 35 kDa were identified in the raw sesame milk sample. Although there was a band near 11 kDa, this band's intensity was less, and it looked more like a smear. In 20- and 30-min plasma-bubbled samples, new bands in the range of 45–65 kDa were visible. In addition, in plasma-treated milk samples below 35 kDa, three distinct bands were visible; it was observed that the middle band was not visible in the case of raw sesame milk sample and 10-min plasma-bubbled sesame milk. After 20-min plasma bubbling, the intensity of the protein bands near 20–25 kDa increased, especially the last two bands in the bottom. At 30-min bubbled sample, band

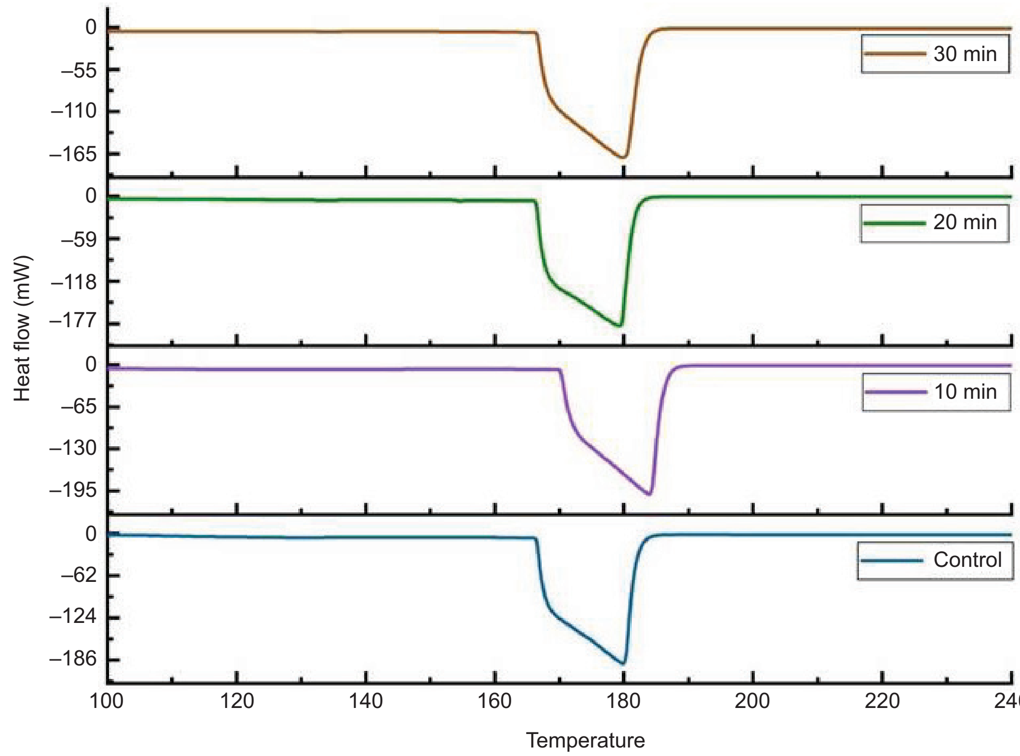

Figure 3 Thermal profile of raw and plasma-processed sesame sample.

Table 1 Thermal parameters assessed by DSC: onset, peak temperature, and enthalpy.

Treatment	Onset temperature (°C)	Peak/denaturation temperature (°C)	Enthalpy (J/g)
Control	165.95	169.65	1360.73
10 min	169.59	173.07	1330.13
20 min	165.83	169.95	1251.41
30 min	166.02	170.79	1240.28

intensities (20–25 kDa) increased such that there was no clear distinction between the bands found in the previous treatments.

Fourier transform infrared spectroscopy

The extracted milk samples were freeze-dried and tested for FTIR analysis. The IR wavelength range of 1600–1700 cm^{-1} was in the Amide I band region that represented five major secondary protein structures, such as *a*-helix, β-turns and sheets, 3_{10}-helix, and random coils. The prominent bands exhibited in sesame were *a*-helix in the wavelength range of 1648–1660 cm^{-1}, β-sheets in the range of 1610–1642 cm^{-1}, β-*turns* in the range of 1660–1680 cm^{-1}, and anti-parallel β-structures in 1680–1700 cm^{-1} range.[7,29] Similar peaks were identified in the FTIR spectra obtained in this study on sesame milk. In the present study, the peaks obtained in the Amide I region were carefully examined using the OriginPro software. Based on the results shown in Table 2, it was concluded that *a*-helix structures increased after plasma treatment. In contrast, the β-helix sheets decreased after plasma treatment. The percentage of *a*-helices was higher in the 20-min plasma-treated sample, compared to the 30-min plasma-bubbled sample. Anti-parallel β-structures were 0% in 20-min plasma-bubbled sample.

Circular dichroism—secondary structure analysis

In this study, raw unprocessed sesame milk showed a spectrum in two positive coordinates and one in negative coordinate; the positive elliptical dichroic bands were observed from 190 to 200.5 nm and 240 to 270 nm whereas negative dichroic band was observed from 200.5 to 240 nm. The upbeat band in the range of 191–193 nm and negative bands in the range of 208–210 and at 222 nm were reported due to the presence of *a*-helices in the sample. Also, the presence of a negative band in the range of 216–218 nm and positive at 195 nm represented the structure of β-sheet in the sample.[30] It was observed that the total *a*-helices increased with plasma treatment, and total β-sheets decreased with an increase in plasma treatment time. Further, the deconvolution of circular dichroism spectra in the CD Pro software based on the method followed by Ghosh et al. was revealed to have consistent results with that of the graphs obtained.[31] Table 3 represents the proportions of *a*-helices, β-sheets, turns, and unordered structures in the control and the plasma-treated samples. It was determined that the contents of *a*-helices were more, compared to that of β-sheets; bubbling of sesame milk increased the regular *a*-helices structures in raw sesame milk (27.13%) to 39%, 29.03%, and 29.9% for 10-,

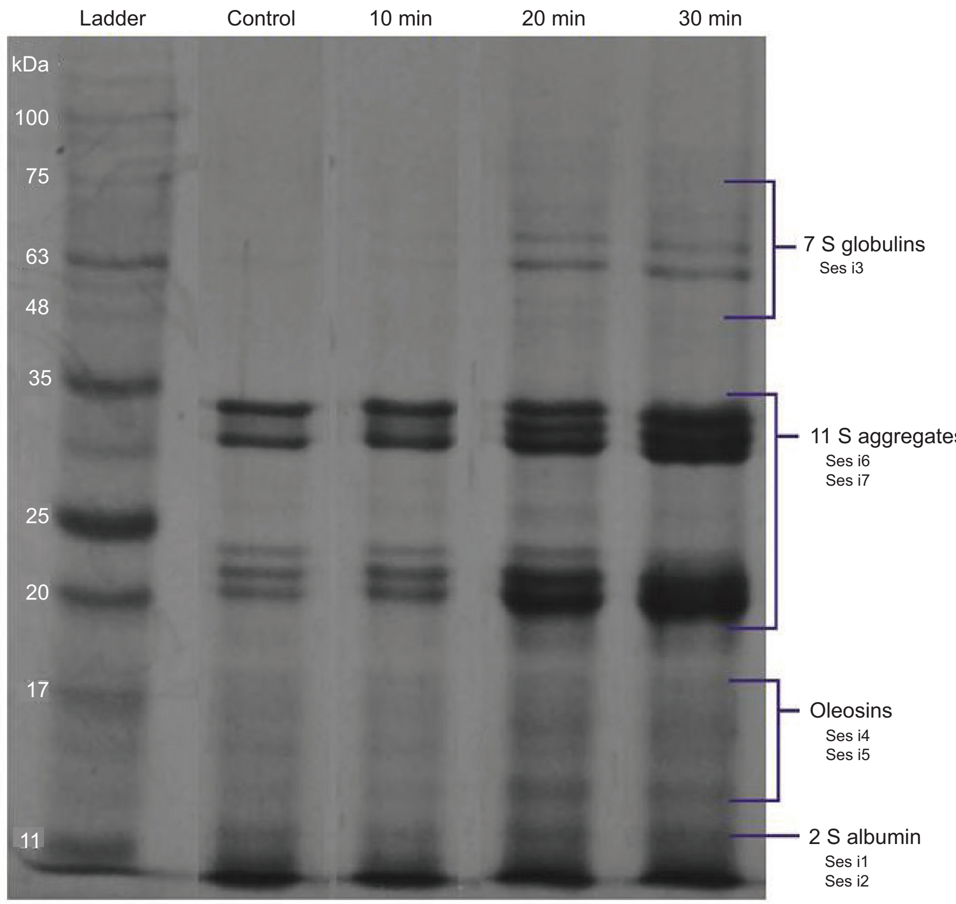

Figure 4 SDS-page protein profile of raw and plasma-processed sesame milk.

Table 2 FTIR analysis of the percentage of secondary structures present in raw and plasma-treated sesame milk samples.

Treatment	α-sheets (%)	β-sheets (%)	β-turns (%)	Anti-parallel β-structures (%)
Control	44.34	8.75	34.25	12.63
10 min	39.1	7.3	35.46	18.07
20 min	77.94	6.77	15.27	0
30 min	60.02	5.69	23.47	10.8

Table 3 Circular dichroism analysis of proteins' secondary structure of raw and plasma-processed sesame milk samples.

Treatment	Regular α-helix	Distorted α-helix	Regular β-sheet	Distorted β-sheet	β-turns	Random coils
Control	27.13	14.3	8.56	8.56	18.23	23.1
10 min	39	20.46	6.2	6.53	11.3	16.43
20 min	29.03	17.66	8.6	7.56	14.83	22.16
30 min	29.9	17.9	8.16	7.73	16.4	20.56

20-, and 30-min plasma-bubbling time, respectively. Regular *α*-helices were more in the case of 10-min plasma-bubbled sample. However, it decreased to around 29% at 20- and 30-min plasma bubbling. Similar results showing increase at 10 min and decrease at 20 and 30 min were observed for distorted *α*-helix. Regular β-sheets increased in the case of 20-min bubbling, and distorted β-sheets decreased for all plasma exposure periods. β-turns and random coils were also reduced after plasma treatment.

Sandwich enzyme-linked immunosorbent assay

The IgE-binding capacity of antigens in the freeze-dried sesame milk sample was evaluated using the specific sandwich-type ELISA test for sesame, as presented in Table 4. The 10-min plasma-bubbled sample showed a 4.21% reduction in IgE binding whereas the 20-min plasma-bubbled sample showed the highest reduction in binding, accounting to 23.1% reduction, compared to the control

Table 4 Changes in allergenicity in terms of reduction in IgE binding in plasma-bubbled samples.

Treatment	Percentage reduction in IgE binding after plasma treatment (%)
10 min	4.21 ± 0.86^{b}
20 min	23.08 ± 4.88^{a}
30 min	-3.69 ± 6.28^{b}

^{a,b,c} values having varying alphabetical numbering are significantly different.

sample (Table 4). However, the plasma-bubbling time of 30 min increased the binding capacity of antigens with IgE antibody; it accounted to 3.7% increase in binding capacity, compared to the control sample.

Discussion

Sesame seeds can cause IgE-moderated allergies in individuals. Proteins in sesame seeds mainly induce IgE-mediated allergies, whereas non-IgE-mediated allergies are caused by lipids.[11] The primary storage proteins (11S globulin, 2S albumin, 7S globulin, and oleosins) present in sesame are potent enough to cause allergies. The significant allergens are further divided into seven subunits such as Ses i 6 and Ses i 7 (11S globulin), Ses i 1 and Ses i 2 (2S albumin), Ses i 3 (7S globulin), and Ses i 4 and Ses i 5 (oleosins).[32] Cold plasma processing of proteins can alter the IgE-binding capacity of an antigen (allergen present in the product) through oxidation of proteins by reorienting the protein structure, or by destroying the epitopes and forming new epitopes.[18] Cold plasma can either decrease or increase the IgE-binding capacity based on the protein conformation. Thus, understanding the characteristics, structure, and IgE-binding capacity of these plasma-treated proteins helps in identifying the nature of sesame allergen and its binding with IgE human antibodies. Before studying the protein structure, it is also necessary to assess the efficiency of plasma equipment in generating reactive species.

Electron paramagnetic resonance spectroscopy uses the paramagnetic behavior of free radicals to detect them. It detects the intensity of microwave absorption by the unpaired electrons present in free radicals under the influence of an applied magnetic field.[33] OH radical is the most crucial and primary reactive species for generating secondary reactive species and is the primary species generated during the splitting of a water molecule.[34] Thus, OH radical was chosen to assess the effect of plasma processing time on reactive species formation. As reactive species are short-lived, spin trap agents are used in general to trap them for longer duration. DMPO, a spin-trapping agent specific for OH radicals, was used in this study to form adducts (DMPO-OH). An increase in intensity at 20 min, compared with 10 min, could be due to the dissolution or formation of more reactive species at higher exposure time. This increase in OH radical proves that the dissolution of reactive species in sesame milk increased with increase in exposure time.[35] This could also be explained by the changes in pH and conductivity with respect to exposure time.[36] However, decrease in intensity at 30 min could be due to the saturation of radicals between 20- and 30-min plasma-bubbling time. Similar results of saturation of DMPO-OH adducts were observed followed by prolonged exposure period.[33,37] Another reason could be the increase in nitric oxide or other nitric acids in milk which could decay DMPO-OH adducts in sesame milk.[38] This proves that reactive species increase with increase in plasma exposure time.

As thermo-assisted changes in the sample correspond to the changes in the sample's proteins and starch, the thermal profile of sesame milk was analyzed using a DSC. It aids in studying the behavior of food material when these are subjected to various changes in temperature and time. While doing so, the equipment measures the heat flow generated in the food material and represents its corresponding changes in the form of a thermogram.[39] The thermogram represents the area of the enthalpy associated with heating process.

The study revealed that the plasma treatment changed the sample's peak temperature and enthalpy. An increase in both onset and peak temperatures in plasma-processed sesame milk, compared to the control sample, revealed the thermal stability of the plasma-treated samples. Similar results of the increase in peak and onset temperatures were observed in previous studies;[40,41] however, there was no drastic change in the temperatures after plasma treatment. Although there were no visible changes in both onset and denaturation temperatures, the enthalpy varied drastically in plasma-processed milk samples (Table 1). The enthalpy, also called the transition heat, represents the proportion of proteins that were not denatured during the heating process.[42] Thus, it was understood that the percentage of proteins that were not denatured in the plasma-treated sesame milk sample was less. Any change in enthalpy denotes that the proteins' thermal stability is altered, which in turn proves that the proteins are oxidized, compared to the control. The study done by Di et al. on sesame reported that the change in enthalpy is an indication of alteration in the stable structure of sesame protein and aggregation.[7] The results observed in the present study were found in accordance with the cold plasma studies conducted on quinoa flour[43] and carnauba wax;[44] the change reported was due to the loss of protein structure or the reduction in starch crystallinity after plasma treatment.

It was inferred from the data obtained from SDS-PAGE analysis that the bands obtained in the present study displayed a typical pattern of native sesame protein based on the previous studies conducted on sesame and sesame protein isolates.[30,45,46] It was observed from the documented electrophoresis gel that there were significant protein bands in the sesame sample, as mentioned above. The bands obtained in raw sesame milk between 20 kDa and 25 kDa and below 35 kDa were identified as of 11S globulin, and the bands observed below or near 11 kDa were of 2S albumin (not prominent).[47] Similar results of protein bands near 25 and 35 kDa were observed in a study on sesame seed germination.[48] In the present study, plasma treatment increased the band intensity near 45–65 kDa, which was identified as that of 7S globulin.[17] The oxidation of proteins by reactive species in plasma could be responsible for changes in the plasma-treated samples.[49] The formation of new bands at higher molecular weight denotes that the protein bands get aggregated to form higher molecular weight proteins. In addition, increase in the intensity of 11S globulin protein bands denoted the same results. 2S albumin, one of the significant sesame allergens, was not found prominently in this sesame milk sample; also, it did not show any variation in plasma-treated samples. 11S globulin is not soluble in water whereas 2S albumin is a water-soluble protein.[50] Thus, the nonavailability of 2S albumin in the present study could be due to the leaching of this protein in water during the soaking of sesame seeds.

In the case of 11S globulin, the intensity of one particular band faded near 25 kDa after plasma treatment. This could be due to the degradation of that band and the formation of new 7S globulin bands. The formation of clusters in 11S aggregates at 30-min bubbled sample could also be due to increase in the formation of aggregates at higher exposure time. 11S globulins are insoluble aggregates; thus, increase in the intensity of these protein bands indicates the generation of more aggregates in the plasma-processed sesame milk. In addition, Plancken et al. reported that at higher exposure time, the hydrophobic residues are liberated and enhanced the interactions of proteins, leading to re-association and cross-linking.[15]

The reactive plasma species, such as OH radical and ozone, have been reported to be responsible for the oxidation of proteins and formation of aggregates in food products.[51] Changes in protein bands could be attributed to the electrostatic interaction of reactive species, formation of disulfide bonds, and interchange of hydrophilic/hydrophobic bonds because of interaction of reactive plasma species with sesame proteins.[52] Formation of reactive species in plasma bubbling unit and its increase with increase in bubbling time, as found using EPR spectroscopy, proved that changes in protein bands with an increase in exposure time was due to the interaction of reactive species with proteins. Similar results of increase in aggregates followed by plasma exposure were observed in the cow milk protein,[53] meat,[54] squid,[55] and tropomyosin.[18] Although there were changes in protein bands, alteration in protein binding can be better understood by studying structural changes in proteins. Secondary structures of proteins are mainly composed of α-helix and β-sheets. The percentage of these structures depends on the nature of the food material and treatments.[56] Thus, FTIR and circular dichroism analyses were conducted to study structural changes in proteins after plasma application in sesame milk samples.

The secondary structure of proteins was studied by analyzing Amide I region in FTIR spectra, as the absorption related to this region matched the vibration of protein mainstay.[57] Also, a particular work on sesame protein isolate's FTIR study reported that the absorption spectra of Amide I region matched the proteins' secondary structures.[7] Similarly, in the present study, we studied Amide I region and found changes in the protein structure after plasma treatment. It was observed that α-helical structures increased after plasma treatment, and β-structures decreased in plasma-treated samples. The data obtained from circular dichroism and calculation using the CD pro software observed that the contents of α-helices were more than that of β-sheets. Therefore, it was inferred that α-helices increased with plasma treatment, and β-sheets decreased with an increase in plasma treatment time. However, the percentage of α-helices decreased at 30-min exposure in FTIR and at 20- and 30-min exposure in circular dichroism in plasma-treated samples; this could be due to the conversion of helical structures to β-sheets at increased plasma processing conditions.

Most of the studies on plasma-treated proteins showed a decrease in helical structure and an increase in sheets.[49,54] This could be due to the nature of the proteins used for analysis and the treatment parameters used. For example, in a study conducted on sunflowers, protein isolate and sodium caseinate were treated simultaneously using a moderate electric field, during which it was observed that β-sheets increased in sunflower protein isolate whereas α-helix increased in sodium caseinate.[58] On the contrary, in another study on plasma treatment of glycinin, it was observed that plasma did not have any significant change in the secondary structure of proteins.[59] This phenomenon proved that the nature of proteins essentially determined the structural changes during treatment. Similarly, one particular study on cold plasma processing of milk casein and whey revealed that the phenomena of change in protein structure depended on the nature of the protein.[53] Plasma processing of whey decreased the α-helix structure whereas a similar treatment on casein increased the α-helix structure. Thus, it was inferred that the cold plasma processing of sesame milk proteins (as a whole) increased α-helix and decreased β-sheets.

An increase in the α-helix structures of sesame (FTIR and circular dichroism data) could be attributed to the increased denaturation (cross-linking or aggregation) of proteins with respect to plasma treatment.[60] These changes revealed that the plasma exposure of sesame milk altered the protein structure. An increase in α-helix denoted a decrease in protein hydrophobicity because of improvement in protein–water interaction.[61] Semwal and Meera reported that increase in α-helix in sorghum protein during microwave treatment was due to aggregation of proteins (formation of disulfide bonds while unfolding proteins).[62] They also stated that water present in the sample could have promoted hydrogen bonding in amino acids cross-linkage. In a study on ultrasonicated whey protein, aggregation of proteins caused by cavitation revealed that β-sheets cross-linked to form aggregates at prolonged ultrasonication time.[63] In the case of plasma, reactive species were reported to change the conformation of proteins and produce more sulfhydryl groups, which, in turn, interacted with one another to form aggregates.[64] Thus, in the present study, action of reactive species in milk (liquid) could be the most appropriate reason for the oxidation of proteins, which in turn promoted aggregation of sesame proteins.

Studies conducted on sesame protein structure proved that denaturation occurred during plasma treatment and changed its structure with change in exposure time. As the processing method changed the conformation of epitopes or the generation of new epitopes (neoepitopes),[65] they could either increase or decrease allergenicity based on changes in protein conformation.[66] Thus, sandwich-type ELISA test was conducted to understand the influence of processed sesame milk on the degree of antigen-binding capacity with IgE antibody. The analysis revealed that the percentage of antigen binding with the sesame-specific IgE antibody was less in plasma-treated samples. Reduction in binding was observed to be more in the 20-min plasma-bubbled sample than in 10- and 30-min bubbling sample. This was inferred in accordance to changes in protein structure. Changes in conformation could be the possible reasons that could have masked desirable epitopes for binding, which in turn decreased IgE binding.[67]

Similar results showing reduction in IgE binding were reported in plasma-treated samples, such as soybean isolate 75%,[27] peanut 43%,[49] β-lactoglobulin 56%,[68] glycinin 91%,[69] and king prawn (tropomyosin) 17.6%.[18] Results demonstrated that changes in proteins because of conformation were the primary reasons for reduction in allergenicity. However, few studies on plasma also reported no evident change in the IgE-binding capacity of cashew nuts[70] as well as casein and whey.[71] It proved that plasma didn't have any impact on some food materials, although results of a few studies revealed that plasma treatment increased the percentage of IgE binding, compared to the control sample. One particular study showed that plasma treatment increased allergenicity in the case of β-lactoglobulin.[53] The study revealed that change in protein structure not only reduced allergenicity but can also increase it; formation of new desirable epitopes for binding or the revealing of more epitopes could be the possible reason for this increase.[56] This could be the reason for increase in allergenicity while treating sesame milk for 30 min. The epitopes formed in 30-min bubbled sample would have favored IgE binding. Results of a study revealed that plasma's effect on allergenicity reduction depended on the processing parameters and nature of the food material used.[72] Another critical factor that contributed to allergenicity reduction was the presence of water in the sample, which was reported to enhance the generation of critical reactive species during the splitting of water molecule, which in turn helped in protein oxidation.[73] Thus, it could be concluded that the water present in sesame milk also contributed to the reduction of allergen-binding capacity. Although plasma has variations in the effects it has on antigen-binding capacity depending on the nature of the food material and process parameters used, this study observed that plasma was the potential element for reducing the allergenicity of sesame milk at a processing time of 20 min. The future studies can use different plasma processing parameters for further studying their effect on proteins.

Apart from studying the nature of protein, it is also quintessential to analyze the effect of plasma on lipid oxidation. Cold plasma has been reported to have negative impact on lipids because of the presence of reactive species, which increase oxidation byproducts and adversely affect the quality of final product.[61,74-76] Thus, because sesame is rich in oil content, the future studies must analyze the plasma-induced oxidation byproducts in sesame milk.

Conclusion

The study on cold plasma treatment of sesame milk proved that plasma caused oxidation of sesame protein, thus reducing the binding capacity of sesame antigen with IgE antibody. SDS-PAGE and DSC analyses confirmed the plasma-induced oxidation of proteins. In contrast, changes in the protein structure were demonstrated by FTIR and circular dichroism secondary structure analyses. The primary reason for increase in oxidation was the interaction of reactive plasma species with sesame protein at a higher bubbling time. The EPR analysis for determining the amount of OH radical produced in plasma-treated sesame milk further proved that the reactive species increased with an increase in plasma bubbling time. This study provided insight into the effect of cold plasma on the oxidation of sesame proteins, which facilitated reduction in the IgE-binding capacity. Although this study manifested that plasma treatment reduced the IgE-binding capacity, the proportion of reduction in binding was less 23%. Thus, it is necessary to conduct further studies on reducing the percentage of binding capacity. Additionally, increase in IgE binding at 30-min bubbling, which could be due to the formation of new epitopes, must also be studied further to reveal exact reasons for the change. The future studies, such as combining this technology with other methods, must be considered to promote reduction in sesame allergenicity.

Conflict of Interest

The consent of all the authors of this paper was obtained for submitting the manuscript, and no potential conflict of interest was declared.

References

1. Hadi J, Brightwell G. Safety of alternative proteins: Technological, environmental and regulatory aspects of cultured meat, plant-based meat, insect protein and single-cell protein. Foods. 2021;10(6):1226. https://doi.org/10.3390/foods10061226
2. Sá AGA, Pacheco MTB, Moreno YMF, Carciofi BAM. Cold-pressed sesame seed meal as a protein source: Effect of processing on the protein digestibility, amino acid profile, and functional properties. J Food Compos Anal. 2022;111:104634. https://doi.org/10.1016/j.jfca.2022.104634
3. Karasakal A. Determination of trace and major elements in vegan milk and oils by ICP-OES after microwave digestion. Biol Trace Elem Res. 2020;197(2):683–93. https://doi.org/10.1007/s12011-019-02024-7
4. Chughtai MFJ, Khaliq A, Zahoor T, Mehmood T, Ahsan S, Liaqat A, et al. Plant protein-based beverages. In: Manickavasagan A, Lim L, Ali A. Plant protein foods. Dordrecht, the Netherlands: Springer; 2022, pp. 251–70. https://doi.org/10.1007/978-3-030-91206-2_9
5. Jardim-Botelho A, de Oliveira LCL, Motta-Franco J, Solé D. Nutritional management of immediate hypersensitivity to legumes in vegetarians. Allergol Immunopathol. 2022;50:37–45. https://doi.org/10.15586/aei.v50iSP1.554
6. Ma X, Wang Z, Zheng C, Liu C. A comprehensive review of bioactive compounds and processing technology of sesame seed. Oil Crop Sci. 2022;7(2):88–94. https://doi.org/10.1016/j.ocsci.2022.05.003
7. Di Y, Li X, Chang X, Gu R, Duan X, Liu F, et al. Impact of germination on structural, functional properties and *in vitro* protein digestibility of sesame (Sesamum indicum L.) protein. Food Sc Technol (LWT). 2022;154:112651. https://doi.org/10.1016/j.lwt.2021.112651

8. Goldberg MR, Appel MY, Nachshon L, Holmqvist M, Epstein-Rigbi N, Levy MB, et al. Combinatorial advantage of Ses I 1-specific IgE and basophil activation for diagnosis of sesame food allergy. Pediatr Allergy Immunol. 2021;32(7):1482–9. https://doi.org/10.1111/pai.13533
9. Giammarioli S, Pastorelli A, Boniglia C, Stacchini P, Silano M. Unintended allergens in prepacked foods with and without precautionary allergen labelling: Preliminary data relative to some recalled food types. Qual Assur Saf Crop Foods. 2019;11(5):465–70. https://doi.org/10.3920/QAS2018.1490
10. Mustafayev R, Civelek E, Orhan F, Yüksel H, Boz AB, Şekerel BE. Similar prevalence, different spectrum: IgE-mediated food allergy among Turkish adolescents. Allergol Immunopathol (Madr). 2013;41(6):387–96. https://doi.org/10.1016/j.aller.2012.05.005
11. Gangur V, Acharya HG. The global rise and the complexity of sesame allergy: Prime time to regulate sesame in the United States of America? Allergies. 2020;1(1):1–21. https://doi.org/10.3390/allergies1010001
12. De Rose C, Patti ML, Gambacorta A, Brancato F, Sopo SM. Oral allergy syndrome by fruit and vegetable PR-10 allergy: Accuracy of in vivo diagnosis. Allergol Immunopathol (Madr). 2021;49(2):129–32. https://doi.org/10.15586/aei.v49i2.28
13. Tzen JTC. Beneficial components in sesame proteins and oil. In: The sesame genome. Dordrecht, the Netherland: Springer; 2021, pp. 59–78. https://doi.org/10.1007/978-3-319-98098-0_3
14. Cheng R, Liao X, Addou AM, Qian J, Wang S, Cheng Z, et al. Effects of “nine steaming nine sun-drying” on proximate composition, oil properties and volatile compounds of black sesame seeds. Food Chem. 2021;344:128577. https://doi.org/10.1016/j.foodchem.2020.128577
15. Plancken I Van Der, Verbeyst L, Vleeschouwer K De, Grauwet T, Husband FA, Lille M, et al. (Bio) chemical reactions during high pressure/high temperature processing affect safety and quality of plant-based foods. Trends Food Sci Technol. 2012;23:28–38. https://doi.org/10.1016/j.tifs.2011.08.004
16. Silva ARA, Silva MMN, Ribeiro BD. Health issues and technological aspects of plant-based alternative milk. Food Res Int. 2020;131:108972. https://doi.org/10.1016/j.foodres.2019.108972
17. Hou L, Chen L, Song P, Zhang Y, Wang X. Comparative assessment of the effect of pretreatment with microwave and roast heating on the quality of black sesame pastes. J Food Sci. 2021;86(12):5353–74. https://doi.org/10.1111/1750-3841.15976
18. Ekezie FGC, Sun DW, Cheng JH. Altering the IgE binding capacity of king prawn (Litopenaeus vannamei) tropomyosin through conformational changes induced by cold argon-plasma jet. Food Chem. 2019;300:125143. https://doi.org/10.1016/j.foodchem.2019.125143
19. Munekata PES, Domínguez R, Budaraju S, Roselló-Soto E, Barba FJ, Mallikarjunan K, et al. Effect of innovative food processing technologies on the physicochemical and nutritional properties and quality of non-dairy plant-based beverages. Foods. 2020;9(3):1–16. https://doi.org/10.3390/foods9030288
20. Olatunde OO, Singh A, Shiekh KA, Nuthong P, Benjakul S. Effect of high-voltage cold plasma on oxidation, physiochemical, and gelling properties of myofibrillar protein isolate from Asian sea bass (Lates calcarifer). Foods. 2021;10(2):326. https://doi.org/10.3390/foods10020326
21. Dharini M, Jaspin S, Jagan Mohan R, Mahendran R. Characterization of volatile aroma compounds in cold plasma-treated milk. J Food Process Preserv. 2022;00:e17059. https://doi.org/10.1111/jfpp.17059
22. Aparajhitha S, Mahendran R. Effect of plasma bubbling on free radical production and its subsequent effect on the microbial and physicochemical properties of coconut neera. Innov Food Sci Emerg Technol. 2019;58:102230. https://doi.org/10.1016/j.ifset.2019.102230
23. Saini CS, Sharma HK, Sharma L. Thermal, structural and rheological characterization of protein isolate from sesame meal. J Food Meas Charact. 2018;12(1):426–32. http://doi.org/10.1007/s11694-017-9655-6
24. Cappozzo JC, Koutchma T, Barnes G. Chemical characterization of milk after treatment with thermal (HTST and UHT) and nonthermal (turbulent flow ultraviolet) processing technologies. J Dairy Sci. 2015;98(8):5068–79. http://doi.org/10.3168/jds.2014-9190
25. Srisuwatchari W, Piboonpocanun S, Wangthan U, Jirapongsananuruk O, Visitsunthorn N, Pacharn P. Clinical and *in vitro* cross-reactivity of cereal grains in children with IgE-mediated wheat allergy. Allergol Immunopathol (Madr). 2020;48(6):589–96. https://doi.org/10.1016/j.aller.2019.11.008
26. Vanga SK, Wang J, Orsat V, Raghavan V. Effect of pulsed ultrasound, a green food processing technique, on the secondary structure and *in vitro* digestibility of almond milk protein. Food Res Int. 2020;137:109523. https://doi.org/10.1016/j.foodres.2020.109523
27. Zhang Q, Cheng Z, Zhang J, Nasiru MM, Wang Y, Fu L. Atmospheric cold plasma treatment of soybean protein isolate: Insights into the structural, physicochemical, and allergenic characteristics. J Food Sci. 2021;86(1):68–77. https://doi.org/10.1111/1750-3841.15556
28. Zhang J, Liu W, Fang L, Gu R, Lu J, Li G. Effect of acid and *in vitro* digestion on conformation and IgE-binding capacity of major oyster allergen Cra g 1 (tropomyosin). Allergol Immunopathol (Madr). 2020;48(1):26–33. https://doi.org/10.1016/j.aller.2019.08.001
29. Achouri A, Boye JI. Thermal processing, salt and high-pressure treatment effects on molecular structure and antigenicity of sesame protein isolate. Food Res Int. 2013;53(1):240–51. http://dx.doi.org/10.1016/j.foodres.2013.04.016
30. Achouri A, Nail V, Boye JI. Sesame protein isolate: Fractionation, secondary structure and functional properties. Food Res Int. 2012;46(1):360–9. http://doi.org/10.1016/j.foodres.2012.01.001
31. Ghosh D, Singh PK, Sahay S, Jha NN, Jacob RS, Sen S, et al. Structure-based aggregation studies reveal the presence of helix-rich intermediate during α-Synuclein aggregation. Sci Rep. 2015;5:1–15. https://doi.org/10.1038/srep09228
32. Pi X, Peng Z, Liu J, Jiang Y, Wang J, Fu G, et al. Sesame allergy: Mechanisms, prevalence, allergens, residue detection, effects of processing and cross-reactivity. Crit Rev Food Sci Nutr. 2022;1–16. https://doi.org/10.1080/10408398.2022.2128031 Epub ahead of print.
33. Barba FJ, Roohinejad S, Ishikawa K, Leong SY, El-Din A Bekhit A, Saraiva JA, et al. Electron spin resonance as a tool to monitor the influence of novel processing technologies on food properties. Trends Food Sci Technol. 2020;100:77–87. https://doi.org/10.1016/j.tifs.2020.03.032
34. Dharini M, Jaspin S, Mahendran R. Cold plasma reactive species: Generation, properties, and interaction with food biomolecules. Food Chem. 2023;405(PA):134746. https://doi.org/10.1016/j.foodchem.2022.134746
35. Mollakhalili-Meybodi N, Yousefi M, Nematollahi A, Khorshidian N. Effect of atmospheric cold plasma treatment on technological and nutrition functionality of protein in foods. Eur Food Res Technol. 2021;247(7):1579–94. https://doi.org/10.1007/s00217-021-03750-w
36. Manoharan D, Stephen J, Radhakrishnan M. Study on low-pressure plasma system for continuous decontamination of milk and its quality evaluation. J Food Process Preserv. 2020;45(2):e15138. https://doi.org/10.1111/jfpp.15138
37. Weiss M, Utz R, Ackermann M, Taran FA, Krämer B, Hahn M, et al. Characterization of a non-thermally

operated electrosurgical argon plasma source by electron spin resonance spectroscopy. Plasma Process Polym. 2019;16(2):1800150. https://doi.org/10.1002/ppap.201800150
38. Reszka KJ, McCormick ML, Buettner GR, Michael Hart C, Britigan BE. Nitric oxide decreases the stability of DMPO spin adducts. Nitric Oxide Biol Chem. 2006;15(2):133–41. https://doi.org/10.1016/j.niox.2006.03.004
39. Ji H, Dong S, Han F, Li Y, Chen G, Li L, et al. Effects of dielectric barrier discharge (DBD) cold plasma treatment on physicochemical and functional properties of peanut protein. Food Bioprocess Technol. 2018;11(2):344–54. https://doi.org/10.1007/s11947-017-2015-z
40. Chen Y, Zhang Y, Jiang L, Chen G, Yu J, Li S, et al. Moisture molecule migration and quality changes of fresh wet noodles dehydrated by cold plasma treatment. Food Chem. 2020;328:127053. https://doi.org/10.1016/j.foodchem.2020.127053
41. Chen G, Dong S, Zhao S, Li S, Chen Y. Improving functional properties of zein film via compositing with chitosan and cold plasma treatment. Ind Crops Prod. 2019;129(29):318–26. https://doi.org/10.1016/j.indcrop.2018.11.072
42. Van de Vondel J, Lambrecht MA, Delcour JA. Impact of wheat gluten on the denaturation of egg white and whey proteins. Cereal Chem. 2021;98(6):1282–7. https://doi.org/10.1002/cche.10468
43. Zare L, Mollakhalili-Meybodi N, Fallahzadeh H, Arab M. Effect of atmospheric pressure cold plasma (ACP) treatment on the technological characteristics of quinoa flour. Food Sci Technol (LWT). 2022;155:112898. https://doi.org/10.1016/j.lwt.2021.112898
44. Romani VP, Olsen B, Pinto Collares M, Meireles Oliveira JR, Prentice C, Martins VG. Cold plasma and carnauba wax as strategies to produce improved bi-layer films for sustainable food packaging. Food Hydrocoll. 2020;108:106087. https://doi.org/10.1016/j.foodhyd.2020.106087
45. Ahmadian-Kouchaksaraei Z, Varidi M, Varidi MJ, Pourazarang H. Influence of processing conditions on the physicochemical and sensory properties of sesame milk: A novel nutritional beverage. Food Sci Technol (LWT). 2014;57(1):299–305. http://doi.org/10.1016/j.lwt.2013.12.028
46. Idowu AO, Alashi AM, Nwachukwu ID, Fagbemi TN, Aluko RE. Functional properties of sesame (Sesamum indicum Linn) seed protein fractions. Food Prod Process Nutr. 2021;3(1):4. https://doi.org/10.1186/s43014-020-00047-5
47. Hassan AB, Mahmoud NS, Elmamoun K, Adiamo OQ, Mohamed Ahmed IA. Effects of gamma irradiation on the protein characteristics and functional properties of sesame (Sesamum indicum L.) seeds. Radiat Phys Chem. 2018;144:85–91. https://doi.org/10.1016/j.radphyschem.2017.11.020
48. de Menezes Mares LF, Passos MC, Menezes CC. Interference of germination time on chemical composition and antioxidant capacity of white sesame (Sesamum Indicum). Food Sci Technol. 2018;38:248–53. https://doi.org/10.1590/1678-457x.20217
49. Venkataratnam H, Sarangapani C, Cahill O, Ryan CB. Effect of cold plasma treatment on the antigenicity of peanut allergen Ara h 1. Innov Food Sci Emerg Technol. 2019;52:368–75. https://doi.org/10.1016/j.ifset.2019.02.001
50. Adeleke VT, Madlala NE, Adeniyi AA, Lokhat D. Molecular interactions associated with coagulation of organic pollutants by 2S albumin of plant proteins: A computational approach. Molecules. 2022;27(5):1685. https://doi.org/10.3390/molecules27051685
51. Panpipat W, Chaijan M. Effect of atmospheric pressure cold plasma on biophysical properties and aggregation of natural actomyosin from threadfin bream (Nemipterus bleekeri). Food Bioprocess Technol. 2020;13(5):851–9. https://doi.org/10.1007/s11947-020-02441-w
52. Koddy JK, Miao W, Hatab S, Tang L, Xu H, Nyaisaba BM, et al. Understanding the role of atmospheric cold plasma (ACP) in maintaining the quality of hairtail (Trichiurus lepturus). Food Chem. 2021;343:128418. https://doi.org/10.1016/j.foodchem.2020.128418
53. Ng SW, Lu P, Rulikowska A, Boehm D, O'Neill G, Bourke P. The effect of atmospheric cold plasma treatment on the antigenic properties of bovine milk casein and whey proteins. Food Chem. 2021;342:128283. https://doi.org/10.1016/j.foodchem.2020.128283
54. Qian J, Wang C, Zhuang H, Nasiru MM, Zhang J, Yan W. Evaluation of meat-quality and myofibrillar protein of chicken drumsticks treated with plasma-activated lactic acid as a novel sanitizer. Food Sc Technol (LWT). 2021;138:110642. https://doi.org/10.1016/j.lwt.2020.110642
55. Nyaisaba BM, Miao W, Hatab S, Siloam A, Chen M, Deng S. Effects of cold atmospheric plasma on squid proteases and gel properties of protein concentrate from squid (Argentinus ilex) mantle. Food Chem. 2019;291:68–76. https://doi.org/10.1016/j.foodchem.2019.04.012
56. Coutinho NM, Silveira MR, Rocha RS, Freitas MQ, Duarte MCK, Quero RF, et al. Cold plasma. In: Galanakis C. Sustainable food processing and engineering challenges. Cambridge, MA: Elsevier; 2021, pp. 109–35. http://doi.org/10.1016/B978-0-12-822714-5/00004-8
57. Ji H, Han F, Peng S, Yu J, Li L, Liu Y, et al. Behavioral solubilization of peanut protein isolate by atmospheric pressure cold plasma (ACP) treatment. Food Bioprocess Technol. 2019;12(12):2018–27. https://doi.org/10.1007/s11947-019-02357-0
58. Subaşı BG, Jahromi M, Casanova F, Capanoglu E, Ajalloueian F, Mohammadifar MA. Effect of moderate electric field on structural and thermo-physical properties of sunflower protein and sodium caseinate. Innovat Food Sci Emerg Technol. 2021;67:102593. https://doi.org/10.1016/j.ifset.2020.102593
59. Liu Z, Zhou Y, Tan Y, Cheng J, Bekhit AE, Mousavi A, et al. Influence of mild oxidation induced through DBD-plasma treatment on the structure and gelling properties of glycinin. Int J Biol Macromol. 2022;220:1454–63. https://doi.org/10.1016/j.ijbiomac.2022.09.127
60. Dreskin SC, Koppelman SJ, Andorf S, Nadeau KC, Kalra A, Braun W, et al. The importance of the 2S albumins for allergenicity and cross-reactivity of peanuts, tree nuts, and sesame seeds. J Allergy Clin Immunol. 2021;147(4):1154–63. https://doi.org/10.1016/j.jaci.2020.11.004
61. Barbhuiya RI, Singha P, Singh SK. A comprehensive review on impact of non-thermal processing on the structural changes of food components. Food Res Int. 2021;149:110647. https://doi.org/10.1016/j.foodres.2021.110647
62. Semwal J, Meera MS. In situ thermal modification of kafirin using infrared radiations and microwaves. J Sci Food Agric. 2022;102(5):1903–11. https://doi.org/10.1002/jsfa.11527
63. Silva M, Zisu B, Chandrapala J. Influence of low-frequency ultrasound on the physico-chemical and structural characteristics of milk systems with varying casein to whey protein ratios. Ultrason Sonochem. 2018;49:268–76. https://doi.org/10.1016/j.ultsonch.2018.08.015
64. Chizoba Ekezie FG, Cheng JH, Sun DW. Effects of mild oxidative and structural modifications induced by argon plasma on physicochemical properties of actomyosin from king prawn (Litopenaeus vannamei). J Agric Food Chem. 2018;66(50):13285–94. https://doi.org/10.1021/acs.jafc.8b05178
65. González-Fernández J, Daschner A, Cuéllar C. Allergenicity of vertebrate tropomyosins: Challenging an immunological dogma. Allergol Immunopathol (Madr). 2017;45(3):297–304. http://dx.doi.org/10.1016/j.aller.2016.08.002

66. Dong X, Wang J, Raghavan V. Critical reviews and recent advances of novel non-thermal processing techniques on the modification of food allergens. Crit Rev Food Sci Nutr. 2020;0(0):1-15. https://doi.org/10.1080/10408398.2020.1722942
67. Li Y, Zhang S, Ding J, Zhong L, Sun N, Lin S. Evaluation of the structure-activity relationship between allergenicity and spatial conformation of ovalbumin treated by pulsed electric field. Food Chem. 2022;388:133018. https://doi.org/10.1016/j.foodchem.2022.133018
68. Liu ZW, Zhang LL, Zhou YX, Tang PP, Tan YC, Cheng JH, et al. Characteristics of cold plasma treatment and enzymatic hydrolysis on IgG/IgE-binding ability of β-lactoglobulin. Food Biosci. 2022;50:102161. https://doi.org/10.1016/j.fbio.2022.102161
69. Liu Z, Zhou Y, Wang F, Tan Y, Cheng J, Bekhit AE, et al. Oxidation induced by dielectric barrier discharge (DBD) plasma treatment reduces IgG/IgE binding capacity and improves the functionality of glycinin. Food Chem. 2021;363:130300. https://doi.org/10.1016/j.foodchem.2021.130300
70. Alves Filho EG, Silva LMA, Oiram Filho F, Rodrigues S, Fernandes FAN, Gallão MI, et al. Cold plasma processing effect on cashew nuts composition and allergenicity. Food Res Int. 2019;125:108621. https://doi.org/10.1016/j.foodres.2019.108621
71. Tammineedi CVRK, Choudhary R, Perez-Alvarado GC, Watson DG. Determining the effect of UV-C, high intensity ultrasound and non-thermal atmospheric plasma treatments on reducing the allergenicity of α-casein and whey proteins. Food Sci Technol (LWT). 2013;54(1):35-41. https://doi.org/10.1016/j.lwt.2013.05.020
72. Venkataratnam H, Cahill O, Sarangapani C, Cullen PJ, Barry-Ryan C. Impact of cold plasma processing on major peanut allergens. Sci Rep. 2020;10(1):1-11. https://doi.org/10.1038/s41598-020-72636-w
73. Pi X, Sun Y, Fu G, Wu Z, Cheng J. Effect of processing on soybean allergens and their allergenicity. Trends Food Sci Technol. 2021;118:316-27. Elsevier. https://doi.org/10.1016/j.tifs.2021.10.006
74. Van Durme J, Vandamme J. Non-thermal plasma as preparative technique to evaluate olive oil adulteration. Food Chem. 2016;208:185-91. http://doi.org/10.1016/j.foodchem.2016.04.007
75. Kulawik P, Alvarez C, Cullen PJ, Aznar-Roca R, Mullen AM, Tiwari B. The effect of non-thermal plasma on the lipid oxidation and microbiological quality of sushi. Innov Food Sci Emerg Technol. 2018;45:412-7. https://doi.org/10.1016/j.ifset.2017.12.011
76. Muhammad AI, Xiang Q, Liao X, Liu D, Ding T. Understanding the impact of non-thermal plasma on food constituents and microstructure—A review. Food Bioprocess Technol. 2018;11(3):463-86. https://doi.org/10.1007/s11947-017-2042-9