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Cold Plasma-Induced Structural Reconfiguration and Allergenicity Modulation in Atlantic Cod: Linking Conformational Shifts to Digestibility and Immunoreactivity

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ABSTRACT: This study explores how atmospheric cold plasma (CP) modulates the nutritional and allergenic properties of Atlantic cod through structural protein alterations. CP at 10 min reduced parvalbumin content by 9.14%, likely via α -helix loss and β -sheet increase (confirmed by FTIR amide I shifts and CD analysis), which may hinder IgE epitope recognition. Antioxidant indices TAC, TFC, and TPC peaked at CP10 (21.56%, 68.48%) and CP30 (14.00%), respectively. Protein content increased by 10.76% (pre-digestion) and 30.20% (post-digestion) at CP20, while peptide release rose by 61.18% and 26.27% at CP30. Despite microstructural and conformational changes, digestibility remained stable. These findings highlight CP as a non-thermal strategy that enhances bioactive and hypoallergenic properties of cod, with potential for broader application to other high-allergen aquatic proteins.

Keywords: Cold Plasma; Atlantic Cod; Protein Structure; Allergenicity; Functional Seafood

1. Introduction

Atlantic cod (*Gadus morhua*) is a widely consumed fish species known for its nutritional value and economic importance. It is popular in the human diet with abundant essential nutrients, including vitamins, polyunsaturated fatty acids, and essential amino acids (Mukherjee et al., 2023). However, fish allergy is among the most prevalent food allergies in both pediatric and adult populations. The primary fish allergen is parvalbumin, which is a small calcium-binding protein with a molecular weight ranging from 11 to 13 kDa (Dasanayaka et al., 2024). To date, through the modification of the structural integrity of allergenic proteins, the mitigation of food allergenicity can be achievable (Fadimu et al., 2023).

In recent years, the exploration of novel food processing (thermal and non-thermal) technologies has garnered significant attention to enhance food quality and safety. Among these, cold plasma is an emerging nonthermal technology mostly applied to microbial decontamination and food sterilization (Perinban et al., 2022b). Through the ionization of inducer gas with electrical discharges, cold plasma consisting of electrons, ions, neutral particles, and free radicals can be obtained (Zhang et al., 2021). While several studies have

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applied cold plasma to crustacean proteins (shrimp, prawn) and soy proteins (Ekezie et al., 2019; Shriver, 2011; Venkataratnam et al., 2019), very few have systematically evaluated its effects on fish allergens, particularly parvalbumin in cod. Our work is the first to integrate structures (FTIR/CD), digestibility, sensory attributes, nutritional properties, and immunoreactivity (ELISA/SDS-PAGE) in Atlantic cod, thereby providing a comprehensive view of cold plasma's impact on a major fish allergen. Hopefully, the cod allergenicity can be reduced to further ensure human health, especially the fish-allergic individuals. The optimized processing technique paves the way for future innovations aimed at enhancing food quality and safety. The implications of this study extend beyond the specific context of Atlantic cod, offering broader insights into the cold plasma application in the food industry.

2. Materials and methods

2.1 Cold plasma system

Indirect cold plasma treatment of generating plasma-activated water by dielectric barrier discharge plasma at atmospheric pressure was applied in this study (Fig. 1). The cold plasma system mainly consists of two copper electrodes placed over a quartz tube. The quartz tube acts as the dielectric barrier to carry water during plasma activation. The discharge gap was kept at 4 mm. The copper electrodes are connected to a high-voltage source (PVM 500, Information Unlimited, USA) with 10-kV (peak-peak) voltage and 20-kHz frequency. The estimated energy input was 0.25 J/cm², calculated from voltage, current, and discharge area. Premixed argon and oxygen gas mixture in 98%:2% proportion was used to produce NTP within the discharge gap. The gas flow rate was maintained at 4 slm (standard liter per minute) and the water flow rate was 500 mL/min. Plasma-activated water was generated by activating 400 mL of distilled water for 20 min based on the optimized time. The PAW exhibited an acidic pH of 3.1, an oxidation–reduction potential (ORP) of approximately +620 mV, hydrogen peroxide (H₂O₂) concentration of ~12 mg/L, and nitrate (NO₃[−]) concentration of ~6 mg/L. These values are consistent with previously reported ranges (Perinban et al., 2022a).

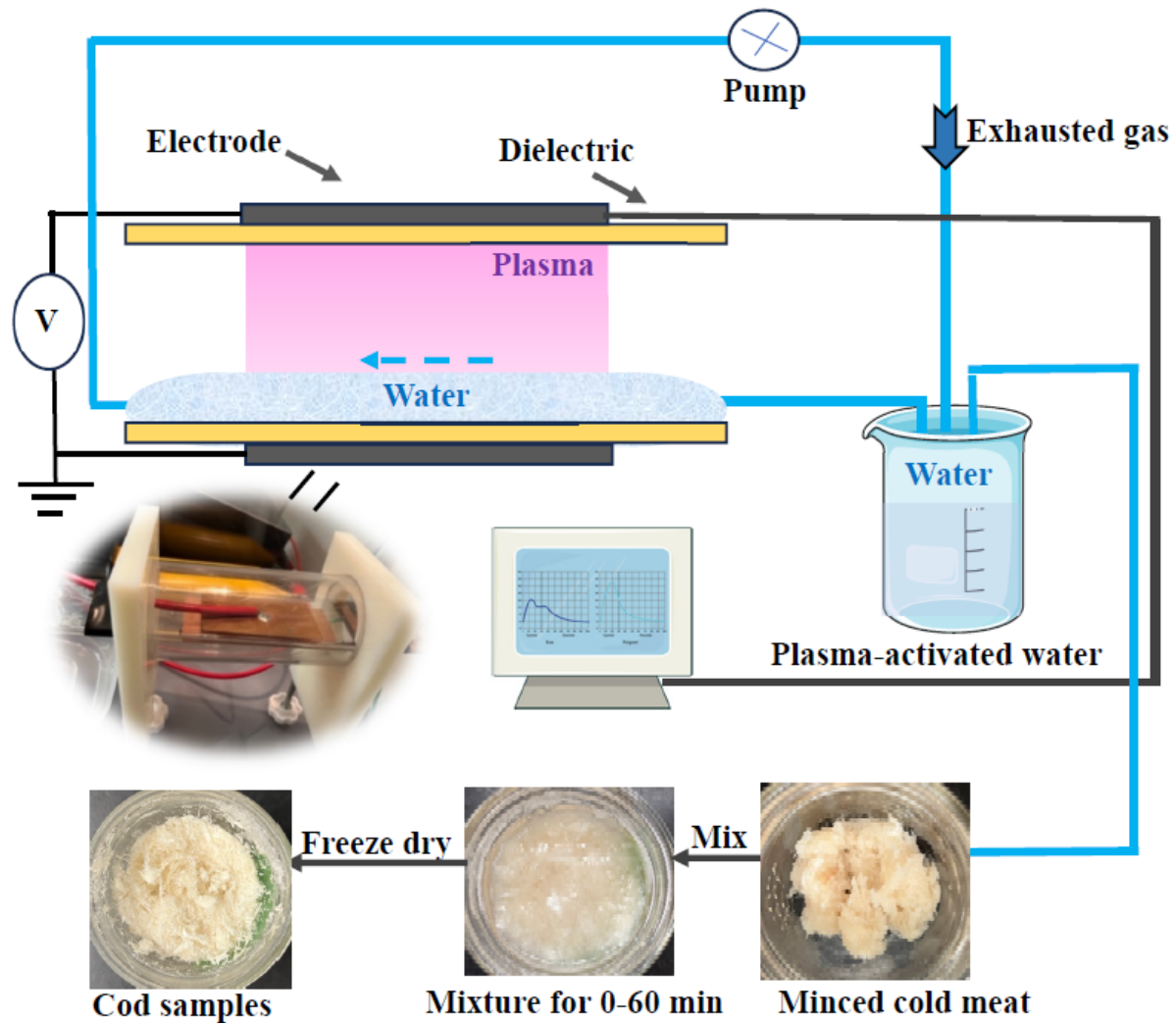


Fig. 1. Schematic diagram of cold plasma system and sample preparation.

2.2 Sample preparation

The Atlantic cod meat used in this study was provided a local seafood market (L'artisan de la mer, Montreal, Canada). A knife mill GRINDOMIX GM 200 (4000 RPM for 90 seconds, 1000W, Retsch, USA) was used to grind and homogenize Atlantic cod fillets into mince. The minced cod was treated by mixing with plasma-activated water by a ratio of 1:1 (per gram/per milliliter). The treatment time durations were 0 min (CP0), 5 min (CP5), 10 min (CP10), 20 min (CP20), 30 min (CP30), and 60 min (CP60), respectively. The equivalent amount of distilled water was used to treat the control group (CP0) for hydration effects. After the treatments, bits of samples were immediately analyzed for color, temperature, pH, and microstructure. The leftover processed samples were subjected to a freeze dryer (7420020, Labconco Corporation, Kansas City, USA) for 48 h. The cod powder was stored at -20 °C until further analysis. All treatments and analyses were performed in triplicates.

2.3 Color attributes

The color attributes of cod samples were determined according to the previous assay described by Dong and Raghavan (2024). The original data were collected eight times by using a colorimeter (CR-300 Chroma,

Minolta, Japan). The total color difference (TCD), chroma (C), hue angle (h), yellow index (YI), and color index (CI), browning index (BI) were calculated based on the formula.

2.4 Total antioxidant capacity (TAC)

The TAC of cod sample was determined by ferric-reducing antioxidant power (FRAP) assay, following the method previously described by Dong and Raghavan (2024). The absorbance was recorded at 593 nm, and the TAC values were calculated with the unit of $\mu\text{mol Fe (II)}/100\text{ mL}$.

2.5 Total flavonoid content (TFC)

The TFC values were determined according to the procedure proposed by Osae et al. (2019). The absorbance was recorded at 510 nm by a microplate reader (Synergy HTX Multi-Mode Reader, BioTek Instruments, Canada). The final results were united by mg of catechin equivalents per gram of dry weight of the cod sample.

2.6 Total phenolic content (TPC)

The TPC contents were measured by following the methodology described by Feng et al. (2021). The absorbance was recorded at 760 nm by using a microplate reader (Synergy HTX Multi-Mode Reader, BioTek Instruments, Canada). The final values were united by mg of gallic acid equivalents per gram of dry weight of the cod sample.

2.7 In-vitro protein digestibility

The two-stage *in-vitro* digestibility of cod proteins was determined by following the previous assay stated by Dong et al. (2021). The freeze-dried cod powder was weighed at 0.2 g and then used for the protein extraction. The BCA assay was utilized to determine the protein contents before the digestion, after pepsin digestion, and after pancreatin digestion. The final results were calculated according to the formula.

2.8 Peptide content

The peptide contents were determined by using the method mentioned by Dong and Raghavan (2024). The absorbance was read at 340 nm. Absorbance values of cod samples were plugged into the standard curve to obtain the concentration of peptides.

2.9 FTIR spectral analysis

The molecular changes in cod sample were evaluated by using Fourier transform infrared (Nicolet Magna 158,750 FTIR, Nicolet Instrument Corp., Madison, WI) spectroscopy. The absorbance in the spectra region from 700 to 4000 cm^{-1} was scanned. FTIR data was collected by OMNIC software (Version 8, Thermo Nicolet Co., Madison, WI) and then analyzed by OriginPro 2022.

2.10 CD spectral analysis

The secondary structure of cod protein was measured by Circular dichroism (CD) spectroscopy. Chirascan (Applied Photophysics, USA) was set up at 23.4 °C. Each sample was prepared with PBS to a concentration of 6 mg/mL and filtered through a 0.22 μM membrane. All Samples were scanned from 190 to

260 nm with a bandwidth of 1 nm, time per point used was 0.5 s and the scanning time for once was 65 s. A cuvette with a path length of 1 mm was filled by 185 μ L of sample for each sample measurement. The final spectrogram was averaged by five accumulated spectra. The DichroWeb (<http://dichroweb.cryst.bbk.ac.uk>) was used for the analysis of protein secondary structures.

2.11 SDS-PAGE analysis

The protein samples were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). An extract of 10 μ L was combined with mercaptoethanol and Laemmli buffer, and then they were heated for 5 minutes at 95 °C. The first lane contained 5 μ L of protein ladders (10–250 kDa) for molecular weight reference, and each lane had 10 μ L of sample. A vertical Mini-PROTEAN® Tetra system (Bio-Rad, USA) was used for electrophoresis at 200 V. After running, the gels were rinsed in water and stained with Bio-Safe™ Coomassie G-250 stain for 60 min, followed by a 30-min water rinse. A Canon EOS Rebel SL2 camera was used to photograph the stained gels.

2.12 ELISA analysis

A sandwich ELISA (Fish Parvalbumin ELISA Kit, Arigo Biolaboratories) was utilized to quantify the parvalbumin content in the cod samples according to protocol. After incubation and washing following microplate procedures several times, the solution color changed from blue to yellow. The absorbances were read at 450 nm by a microplate reader (Synergy HTX Multi-Mode Reader, BioTek Instruments, Canada).

2.13 SEM observation

Microstructural changes of cod samples were observed by a Scanning Electron Microscope (SEM) (TM3000, Hitachi High-Technologies Corporation., Tokyo, Japan) was applied to observe microstructural changes in cod samples. A piece of freeze-dried cod sample was transferred to the measuring platform for observation. For microstructural visualization, the images were captured when they were magnified 100 times.

2.14 Optical microscope

The cod samples (15 μ L) was diluted and stained by using toluidine blue solution (0.1%, Sigma-Aldrich, Canada) on the glass slide for 2 min. An optical microscope (Leica DM500, Leica Microsystems Inc., Canada) and imaging software (Leica LAS EZ, Leica Microsystems Inc., Canada) were used to observe the microstructural changes and image capture (magnification at 4 times) as described by Dong et al. (2020).

2.15 Statistical analysis

The analysis of variance (ANOVA) feature of the SPSS program (IBM SPSS Statistic, Ver. 29.0.0.0) was used to examine the experimental data. The means were separated using the Duncan, multiple range test, and significance was established at $p \leq 0.05$.

3. Results and Discussions

3.1 Color, pH and temperature

Table 1 presents the effects of CP treatment on cod fillets, as evidenced by changes in color parameters, pH levels, and temperature over different treatment durations. The L^* values, indicative of lightness, varied significantly across treatments, with CP20 showing the highest lightness (77.85 ± 0.30) and CP5 the lowest (59.71 ± 2.20). This suggests that CP treatment can cause noticeable changes in the lightness of cod fillets, potentially due to protein denaturation or surface dehydration (Huang et al., 2019). The a^* values, representing the green-red chromatic scale, remained statistically consistent across treatments, indicating that redness/greenness was not significantly affected by cold plasma. The b^* values, representing the blue-yellow chromatic scale, showed some variability, with CP10 having the lowest b^* value, indicating less yellowness. The TCD and Chroma (C) values highlight the overall color changes due to CP treatment. CP5 showed the most significant color change (TCD: 10.03 ± 0.70), which might be attributable to surface oxidation (Ekielski et al., 2013). The consistency in Chroma across most treatments suggests that the intensity of color remained relatively constant, despite variations in lightness and yellowness. The Hue (h) and Yellow Index (YI) further support these observations. The Hue varied slightly, indicating minor changes in the color tone, while the Yellow Index showed notable differences, particularly at CP30, which had the highest YI value (13.40 ± 0.12), suggesting increased yellowness.

Table 1. Changes in the physiochemical properties (color, pH, temperature TAC, TFC, and TPC) of cod samples treated by cold plasma.

		CP0	CP5	CP10	CP20	CP30	CP60
Color attributes	L	70.08 ± 1.79^b	59.71 ± 2.20^d	69.35 ± 2.83^b	77.85 ± 0.30^a	64.46 ± 3.28^c	58.37 ± 1.95^d
	a	-1.12 ± 0.25^a	-1.08 ± 0.14^a	-1.16 ± 0.02^a	-1.44 ± 0.16^a	-1.59 ± 0.16^a	-0.71 ± 0.10^a
	b	5.84 ± 0.82^a	4.92 ± 0.71^{ab}	4.19 ± 0.44^b	5.83 ± 0.61^a	6.41 ± 0.64^a	2.10 ± 0.95^c
	TCD	-	10.03 ± 0.70^b	6.58 ± 0.62^d	7.60 ± 0.48^c	4.16 ± 0.44^c	11.83 ± 0.17^a
	C	5.95 ± 0.80^a	3.67 ± 0.86^b	6.59 ± 0.89^a	6.01 ± 0.63^a	6.60 ± 0.66^a	2.22 ± 0.92^b
	h	-1.38 ± 0.05^b	-0.61 ± 0.25^a	-0.83 ± 0.78^{ab}	-1.33 ± 0.01^b	-1.23 ± 0.01^{ab}	-1.21 ± 0.12^{ab}
	CI	2.74 ± 0.15^{ab}	2.23 ± 0.01^c	2.48 ± 0.04^{bc}	2.63 ± 0.17^{abc}	2.96 ± 0.30^{ab}	3.10 ± 0.48^a
	YI	11.53 ± 1.32^b	11.29 ± 1.67^b	9.07 ± 1.35^c	11.07 ± 0.89^b	13.40 ± 0.12^a	5.07 ± 0.85^d
pH		6.73 ± 0.01^a	6.07 ± 0.03^c	6.29 ± 0.01^d	6.44 ± 0.02^c	6.60 ± 0.01^b	6.29 ± 0.00^d
Temperature (°C)		24.23 ± 0.06^b	25.20 ± 0.10^a	24.03 ± 0.06^{bc}	24.10 ± 0.10^{bc}	23.93 ± 0.32^c	24.17 ± 0.15^{bc}
TAC ($\mu\text{mol Fe(II)}/100 \text{ mL}$)		177 ± 2.61^c	183.5 ± 4.29^{bc}	215.17 ± 4.95^a	186.17 ± 3.79^b	191.5 ± 5.77^b	147.67 ± 6.01^d
TFC (mg/g)		1.65 ± 0.16^b	2.55 ± 0.53^a	2.78 ± 0.73^a	2.11 ± 0.06^{ab}	2.33 ± 0.48^{ab}	1.85 ± 0.1^{ab}
TPC (mg/g)		0.5 ± 0.08^{ab}	0.43 ± 0.07^{ab}	0.46 ± 0.11^{ab}	0.5 ± 0.04^{ab}	0.57 ± 0.08^a	0.4 ± 0.01^b

Note: Different letters within a row indicate significant differences ($p < 0.05$) by Duncan's multiple range test.

The pH levels demonstrated a downward trend with increased treatment duration, with CP5 showing the lowest pH (6.07 ± 0.03). This could be attributed to chemical changes in the fish tissue, possibly due to acidic compounds generated during plasma treatment. The temperature of the cod also varied with treatment duration, but not in a consistent pattern. CP5 showed the highest temperature (25.20 ± 0.10 °C), which might influence biochemical reactions in the fish tissue.

3.2 Total antioxidant capacity (TAC), total flavonoid content (TFC) and total phenolic content (TPC)

The treatment of cod with cold plasma for varying durations (0–60 min) yielded notable changes in the TAC, TFC, and TPC (Table 1). The TAC values exhibited a significant increase, with the highest value observed in the CP10 sample (215.17 ± 2.95 n/umol/100mg), indicating a peak in antioxidant activity with a

maximum increase of 21.56%. The findings demonstrate that cold plasma treatment can affect the bioactive compound profiles in cod. Similarly, Farias et al. (2020) revealed that plasma (200 Hz) significantly increased the antioxidant capacity of apple juice. The increase of TAC at CP10 could be attributed to the plasma-induced formation of antioxidant compounds or the release of bound phenolic compounds (Yu & Beta, 2015).

The TFC fluctuated with a maximum value recorded at CP10 (2.78 ± 0.73 mg/g) with an increase of 68.48%, followed by a slight decrease with longer treatment durations. This pattern indicates a time-sensitive response of flavonoids to plasma exposure. The initial increase in flavonoid content could result from the breakdown of complex compounds into simpler flavonoid structures. However, prolonged exposure seems to lead to a gradual degradation or loss of these compounds (Hemmati et al., 2021).

The TPC results demonstrated a slight fluctuation but were almost stable across different treatment times, with the highest value observed at CP30 with an increase of 14.00%. A similar increase of TPC was observed in the blueberry (Ji et al., 2020) and avocado (Batista et al., 2021), while a similarly slight decrease was observed in the acerola juice (Fernandes et al., 2019). Liao et al. (2018) revealed that the decreased phenolic values were probably due to the destroyed structures of the aromatic ring because the phenolics are vulnerable to ozone assaults. Based on the TAC, TFC, and TPC results, the optimal treatment time appears to be CP10, balancing the enhancement of antioxidant capacity and flavonoid content while maintaining the phenolic content.

3.3 *In-vitro* protein digestibility and peptides

The effects of cold plasma treatment on the fluctuation of total soluble protein content and peptide generation were investigated (Table 2). Before the digestion, compared with that of CP0, protein content showed a decreasing trend at CP5 (46.97%), CP10 (43.80%), CP30 (5.26%), and CP60 (11.13%). However, protein content observed before and after a two-stage digestion process could be maximally enhanced by 10.76% and 30.20% at CP20. The peptides exhibited a decrease in both CP5 (42.57%) and CP10 (46.55%). However, peptide concentrations after 20 min showed an increase at CP20 (16.29%), CP30 (61.18%), and CP60 (2.12%). The peptide content before and after this digestion process could see its maximum boost by 61.18% and 26.27% at CP30. The two-stage digestion was *in vitro* simulated gastric and intestinal digestion, corresponding to enzymes of pepsin and pancreatin, respectively. The IVPD values after pepsin digestion fluctuated from 21.71–32.52%. After pancreatin digestion, IVPD values showed an overall decrease in comparison to CP0 (58.02%). The lowest IVPD was found at CP10 (35.32%), followed by CP5 (50.91%), CP30 (53.42%), CP20 (56.43%), and CP60 (56.50%). Kheto et al. (2023) found that the IVPD of guar seed flour increased maximum of 2.9 % after being treated by a plasma reactor (5–20 min) at different power levels (10 and 20 kV). This might be because cold plasma inhibited enzymatic digestibility by inducing the development of complex protein structures (Kheto et al., 2023). Wang et al. (2023) revealed that the IVPD rose by 11.93% in chickpea protein treated by atmospheric pressure plasma jets. The treatment altered the secondary and tertiary structures of chickpea protein, which caused the side chains of amino acids to unfold. This structural change made a large number of cleavage sites visible, improved protease binding and

ultimately increased IVPD (Mollakhalili-Meybodi et al., 2021). Moreover, Dabade et al. (2023) reported that cold atmospheric pressure non-thermal needle electroplating plasma treatment decreased the trypsin inhibitor activity of soybeans, therefore increasing IVPD. However, long-term atmospheric pressure plasma jets can also trigger a decreased IVPD. This can be explained as the disulfide bond formation causing protein aggregation and the absence of proteinase binding sites (Wang et al., 2023).

Table 2. Determination of protein and peptide content (before/ after pepsin/ after pancreatin digestion) and IVPD values.

Treatment (min)	Protein content (mg/g)			Peptide content (mM)			IVPD (%)	
	Before digestion	After pepsin digestion	After pancreatin digestion	Before digestion	After pepsin digestion	After pancreatin digestion	After the first stage	After the second stage
CP0	90.34±1.60 ^b	63.90±2.45 ^b	33.48±2.62 ^c	3.22±0.41 ^b	2.81±0.13 ^b	6.32±1.17 ^b	29.27	58.02
CP5	47.91±0.48 ^c	29.93±2.25 ^c	23.52±0.70 ^d	1.85±0.06 ^c	1.61±0.22 ^c	3.67±0.46 ^{cd}	37.52	50.91
CP10	50.77±1.74 ^c	39.28±3.77 ^d	32.84±0.87 ^c	1.72±0.14 ^c	1.62±0.13 ^c	3.04±0.72 ^d	22.63	35.32
CP20	100.06±4.09 ^a	78.34±1.66 ^a	43.59±3.82 ^a	3.75±0.51 ^b	3.46±0.49 ^a	4.55±0.68 ^c	21.71	56.44
CP30	85.59±3.84 ^c	65.21±2.24 ^b	39.86±4.09 ^{ab}	5.19±0.32 ^a	3.57±0.37 ^a	7.98±0.48 ^a	23.81	53.42
CP60	80.28±2.19 ^d	52.71±5.80 ^c	34.92±5.76 ^{bc}	3.29±0.5 ^b	3.55±0.48 ^a	7.58±0.39 ^a	34.34	56.50

Note: Different letters within a column indicate significant differences ($p < 0.05$) by Duncan's multiple range test.

3.4 FTIR spectroscopy and CD spectroscopy

The FTIR data revealed the molecular changes, involving fatty acids, proteins, polysaccharides, and other compounds (Dziuba et al., 2007). As shown in Fig. 2a, in the fatty acid region ($3100\text{--}2800\text{ cm}^{-1}$), notable differences in peak intensities were observed across samples. The CP10 and CP20 samples showed an increased absorption, indicating possible alterations in the fatty acid composition or structure due to plasma treatment. The CP30 and CP60 samples, however, exhibited less pronounced changes, suggesting a threshold beyond which plasma treatment does not significantly alter fatty acids (Veetil et al., 2023). In the amide region ($1800\text{--}1500\text{ cm}^{-1}$), crucial for protein analysis, it revealed distinct variations in the amide I and II bands. The CP5 and CP10 samples exhibited shifts in peak positions, possibly indicating changes in protein secondary structures, such as alterations from α -helix to β -sheet. The minimal changes at CP0, CP20, CP30, and CP60 suggest that plasma treatment might have a non-linear effect on protein structure. In the mixed region ($1500\text{--}1200\text{ cm}^{-1}$), it showed complex patterns due to overlapping signals from proteins, fatty acids, and phosphates (Ma'alifah et al., 2022). A gradual shift in peak intensity and position was evident in samples treated for longer durations (CP20, CP30, and CP60), hinting at cumulative molecular alterations due to prolonged plasma exposure. In the polysaccharide region ($1200\text{--}900\text{ cm}^{-1}$), it displayed significant changes, especially in CP10 and CP20 samples. These changes might be attributed to plasma-induced modifications in carbohydrate structures or interactions between carbohydrates and other macromolecules (Szymanska-Chargot et al., 2015). In the “fingerprint region” ($900\text{--}700\text{ cm}^{-1}$), known for its unique absorption patterns, it indicated specific molecular changes in the samples. The CP10 sample showed the most distinct pattern, suggesting that this treatment duration led to unique molecular rearrangements or formations (Kozisek et al., 2023).

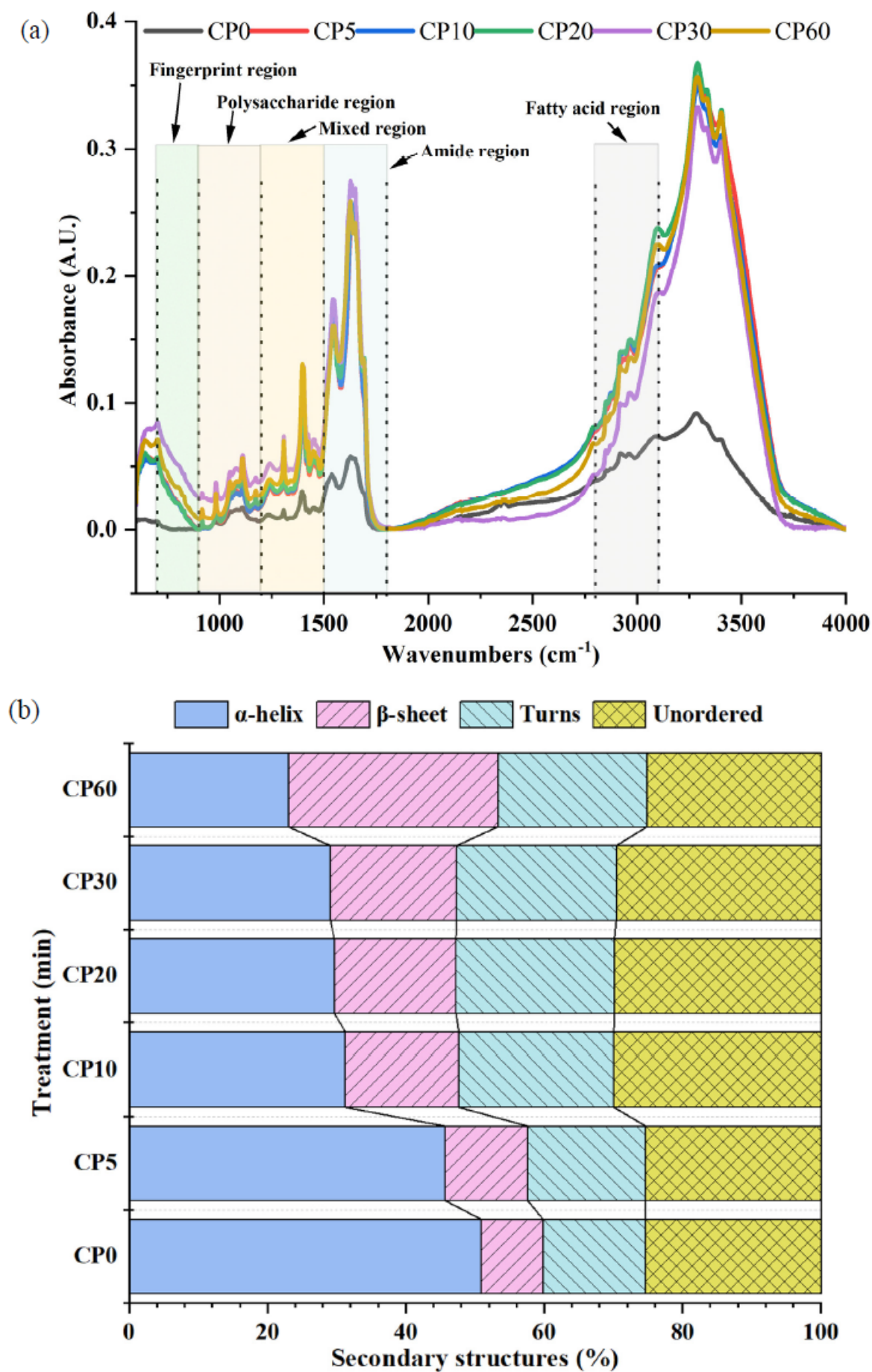


Fig. 2. Molecular changes determined by FTIR spectra (a) and the percentage changes of protein secondary structure by CD spectra (b).

The FTIR results reveal that cold plasma treatment can induce significant molecular modifications in cod, with varying impacts based on treatment duration. The distinct changes at the CP10 sample across multiple spectral regions suggest a threshold at which plasma treatment induces maximum molecular alterations. This could be due to an optimal combination of factors such as reactive species generation, electromagnetic effects, and thermal changes at this specific plasma exposure duration. In contrast, the less pronounced changes at the CP0, CP5, CP20, CP30, and CP60 samples indicate that the effects of plasma treatment are not directly proportional to the duration of exposure. This non-linear response highlights the complexity of plasma-protein interactions and underscores the importance of optimizing treatment conditions to achieve desired molecular modifications without compromising the nutritional and structural integrity of seafood products.

The CD analysis provided insightful information about the secondary structural changes of cod protein. The variations in the percentages of α -helix, β -sheet, turns, and unordered structures are shown in Fig. 2b. With the increased cold plasma duration, α -helix content decreased while β -sheet and turns increased with relatively stable unordered structures. These results suggested plasma can induce conformational changes in the cod protein. Decreased α -helix content could be attributed to possibly oxidative modifications or the hydrogen bond breakage that stabilizes the α -helix structure (Zhang et al., 2020). The increase in β -sheet and turns might be indicative of protein aggregation or unfolding processes, common in stress responses. Protein aggregation often involves a transition from α -helix to β -sheet structures (Housmans et al., 2023). The relatively stable proportion of unordered structures is due to a limited impact of cold plasma on the unordered or random coil regions of the cod protein.

These conformational rearrangements are not only indicative of protein unfolding and aggregation but may also influence allergenicity. Specifically, the transition from α -helix to β -sheet structures could lead to the masking or structural alteration of IgE-binding epitopes, thereby hindering antibody recognition. Such structural changes provide a plausible mechanistic explanation for the reduced parvalbumin detectability observed by ELISA after CP treatment.

3.5 Allergenicity

ELISA analysis was used to determine the allergenicity of cod parvalbumin treated with cold plasma (Fig. 3a). The concentration of parvalbumin at CP0 (1.493 ug/mg) is the highest. After cold plasma treatment, CP10 reached the lowest allergenicity with a decrease of 9.14%, followed by CP60 (8.64%), CP20 (6.07%), CP5 (5.44%), and CP30 (4.38%). Compared with CP0, a slight decrease at CP5 and a more pronounced decrease at CP10 suggest that cold plasma treatment can reduce allergenic protein content. However, the subsequent increases at CP20 and CP30 indicate that the relationship between plasma treatment time and parvalbumin content is not linear and may be influenced by other factors such as protein denaturation, aggregation, or conformational changes that expose or hide epitopes recognized by the ELISA. Followed by another decrease at CP60, the allergenicity showed a significant fluctuation in the treated cod samples. The prolonged treatment seems to further diminish the detectability of allergenic proteins. This reduction is

probably due to extensive protein degradation, which could lead to the loss of epitopes or the creation of aggregates that are not readily detectable by ELISA.

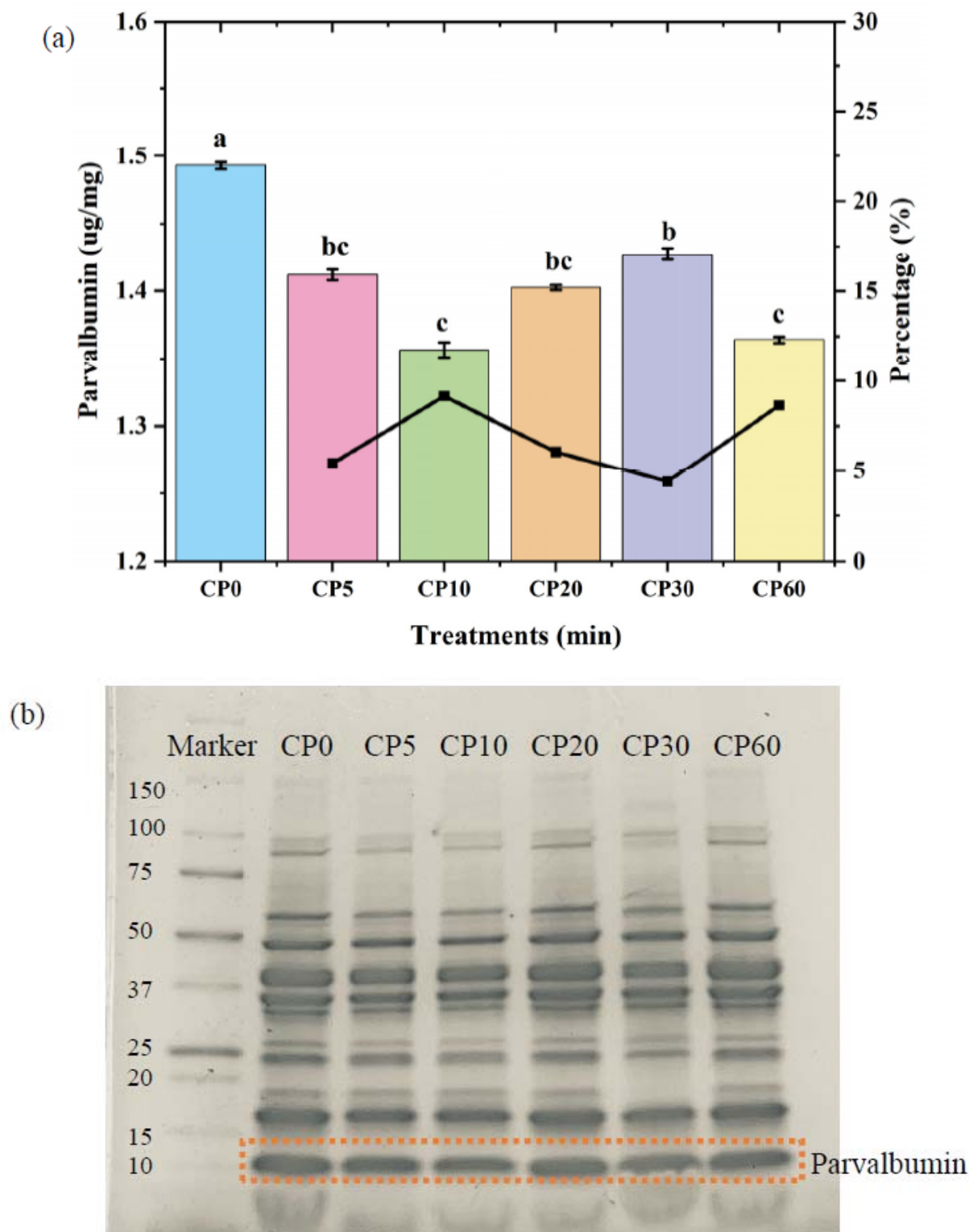


Fig. 3. ELISA analysis of parvalbumin content (a) and SDS-PAGE result (b) cod samples treated by cold plasma.

Venkataratnam et al. (2020) reported that cold plasma (52 kHz, 32 kV, and 118 s) reduced the antigenicity of Ara h 1 by up to 65% and Ara h 2 by up to 66% from dry, whole peanut and defatted peanut flour by using competitive ELISA. Ng et al. (2021) revealed that cold plasma (5-8kV, 25kHz, and 0-30 min) decreased the antigenicity of casein and α -lactalbumin, whereas increasing the antigenicity of β -lactoglobulin

in bovine milk. The decreased allergenicity may be owing to the cold plasma treatments causing the conformational structure to have changed and released hydrophobic or hydrophilic residues that allow antigenic epitopes to be destroyed or reoriented. The increased allergenicity might be due to new epitopes generated after cold plasma treatment, which further increased IgE binding potential (Sathe et al., 2016). In comparison to these studies, the lower reduction in Atlantic cod's allergenicity may be due to the indirect PAW method and cod parvalbumin's structural stability. The optimization of plasma parameters (e.g., higher voltage, different gas ratios, direct exposure) may further enhance allergenicity reduction, which should be done in follow-up work.

Moreover, SDS-PAGE profiles of cod proteins treated by cold plasma treatment are shown in Fig. 3b. The image suggests that the lower band intensity was observed in cold plasma-treated samples compared with the CP0, which corresponded to the ELISA results. This probably correlates with decreased allergenic protein content due to cold plasma treatment. The band located approximately at 10 kDa is cod parvalbumin, with the thinnest band in CP10. However, the band intensity between the treated cod samples was not visibly changed. Similarly, Ng et al. (2021) found that cold plasma (30 min) did not change the band intensity of β -lactoglobulin in milk. Zhou et al. (2016) also reported that the band intensity of bovine serum albumin remained unchanged after cold plasma treatment. This suggests that plasma exposure could not have a significant effect on the cod allergenicity and slight total protein degradation occurred (Venkataratnam et al., 2019).

It should be emphasized that the decrease in parvalbumin concentration observed by ELISA primarily reflects changes in detectable protein levels and thus indicates a potential reduction in allergenicity. However, ELISA alone does not directly confirm decreased IgE binding capacity, which is a more clinically relevant indicator of immunoreactivity. Moreover, in the present study allergenicity was only evaluated prior to simulated digestion, whereas the residual IgE-binding capacity after gastrointestinal digestion would provide more practical insights. The digestibility and peptide-release data already suggest that CP-induced structural reconfiguration may influence epitope exposure during digestion. Nevertheless, future studies will incorporate *in-vitro* digestion followed by IgE immunoblotting assays using sera from fish-allergic patients to directly validate whether these structural alterations indeed translate into reduced immunoreactivity.

3.6 Microstructure

The impact of cold plasma on the morphology obtained by the optical microscope and SEM of cod samples is illustrated in Figs. 4 & 5. The optical microscope images showed a comparison among cod samples with and without cold plasma treatment. In Fig. 4, the CP0 had a smooth shape and a clearer blue color, whereas the treated cod samples were somewhat damaged with a deformed shape and brown color. These results are consistent with the SEM results. In Fig. 5, the surface matrix of the CP0 was compact and tightly packed. After the cold plasma treatment, most samples showed a slightly rough surface, especially in CP10 (indicated by yellow circles). The CP10 had increased surface flaws and crinkled irregular forms, all of which pointed to the deterioration of the polymeric chains (Sadeghi et al., 2021). This could be explained by protein

molecules being broken up and the sample surface being looser due to electrons, ions, and other high-energy particles during the discharge process (Yu et al., 2020). Meanwhile, the marked structural destruction observed in CP10 suggests a critical threshold in cold plasma duration at which protein structures undergo significant alteration. Cold plasma treatment either below or above a 10-min duration does not significantly alter protein structures in cod. In the CP10 sample, the extent of structural unfolding and subsequent exposure of hydrophobic regions may reach a critical level. This can lead to the aggregation of unfolded proteins, as hydrophobic regions from different molecules interact and bind together. Such aggregation is often irreversible and results in the formation of large protein complexes (Khanikar et al., 2022). In contrast, shorter or longer treatment times (as in CP0, CP5, CP20, CP30, and CP60) might not induce sufficient unfolding or might lead to other types of modifications that do not favor aggregation to the same extent.

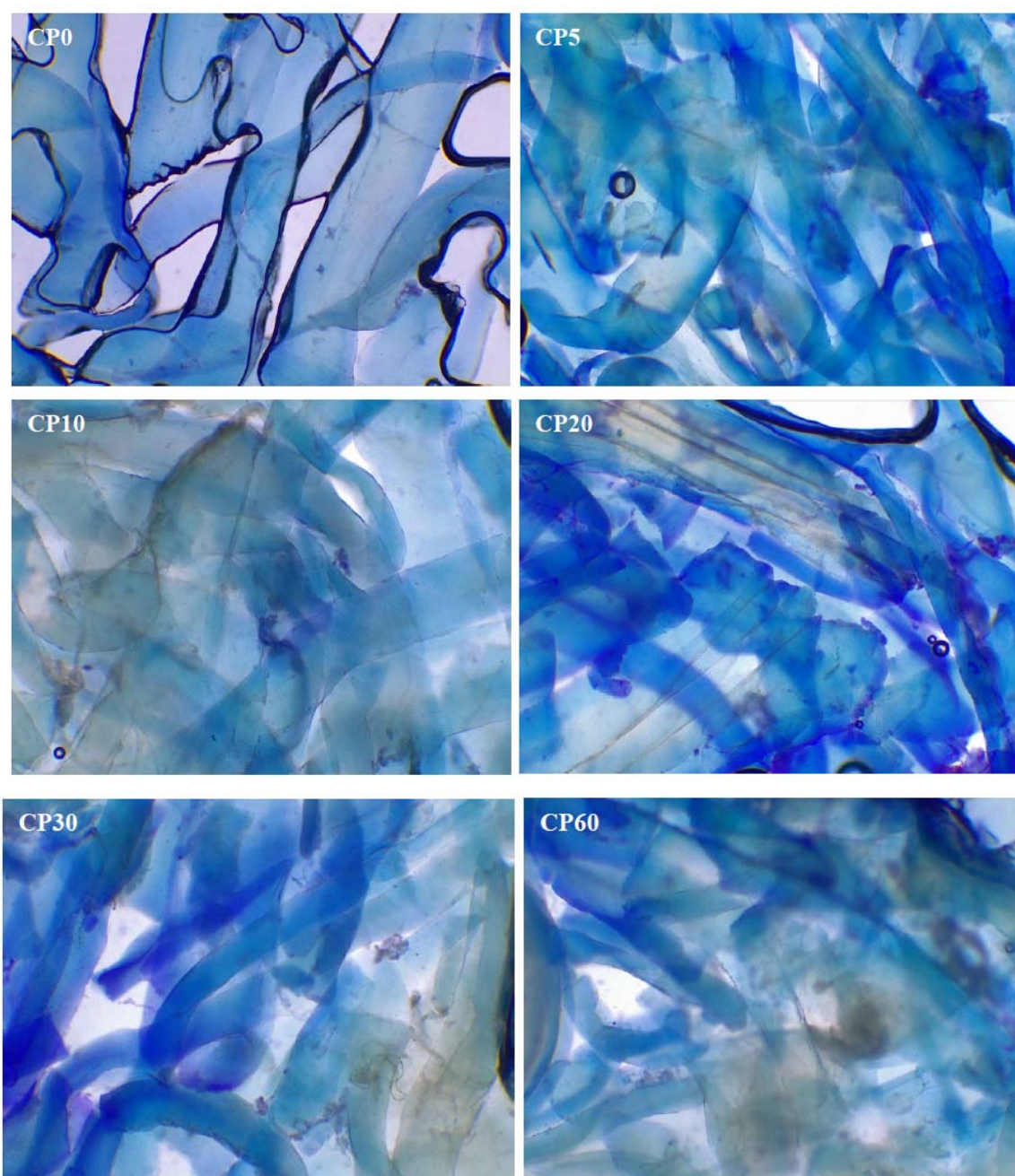


Fig. 4. Optical microscope images of cod samples treated by cold plasma (magnification of 4 times, approximate scale bar = 100 μm).

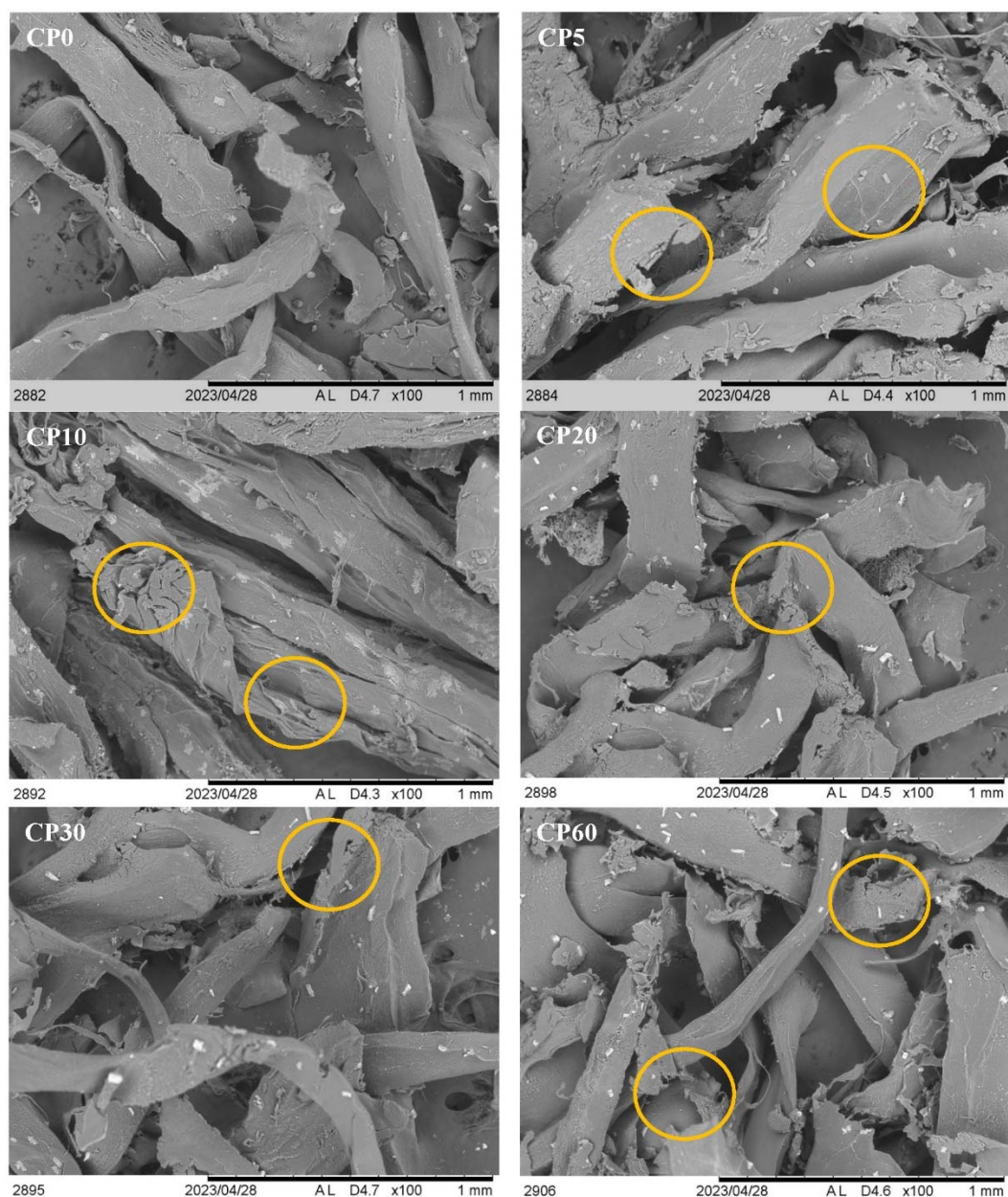


Fig. 5. SEM images of cod samples treated by cold plasma (magnification of 100 times, approximate scale bar of 20 μ m).

4. Conclusions

This investigation illustrates the profound effects of atmospheric cold plasma treatment, as a revolutionary method, for enhancing the nutritional attributes and reducing the allergenic properties of Atlantic cod. The findings reveal a significant reduction in cod allergenicity by 9.14%, alongside noteworthy alterations in the protein and peptide compositions, which were observed to increase notably after a two-stage digestion process. These modifications are critical for the advancement of dietary management practices and for addressing the growing concerns surrounding food allergies. The research also demonstrates an impressive stability in the digestibility of the treated samples, indicating the robustness and reliability of cold plasma treatment. The increased levels of TAC, TFC, and TPC further emphasize the potential of cold plasma to substantially enhance the quality of food products. Ultimately, the results of this study not only augment our understanding of the impact of cold plasma on food but also underscore its vast

potential as a tool for improving food safety, nutrition, and allergenicity profiles. This work sets a precedent for future research in food science, offering a promising direction for the development of innovative strategies in the management of food allergies.

Conflict of Interest

The authors declare no conflict of interest reported in this paper.

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