



Effect of Cold Plasma Treatment on Phenolic Content and Antioxidant Capacity of *Aronia melanocarpa* Extract and Wheat Grass Interaction

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Abstract. This study investigated the effects of wheat grass (WG), *Aronia melanocarpa* extract (AME) and cold plasma (CP) treatments on total phenolic content (TPC), radical scavenging capacity (DPPH) and reducing power (CUPRAC). The findings showed that the AME + CP group had the highest bioactive potential. The TPC value of this group was 64.938 mg GAE/g, which increased by approximately 40% compared to AME without CP treatment. The mixture of WG + AME + CP also showed that CP increased the preservation of phenolic compounds and the extraction efficiency. DPPH activity was determined as 42.626 $\mu\text{mol TE/g}$ in the AME + CP group, and it was observed that CP preserved its radical scavenging capacity. CUPRAC value was 158.422 $\mu\text{mol TE/g}$, which increased by 5% compared to the group without CP treatment. According to ANOVA analysis, the differences were statistically significant ($p < 0.001$), and the high effect size ($\eta^2 > 0.98$) supported the strong effect of CP. High Performance Liquid Chromatography (HPLC) analyses showed that chlorogenic acid and cyanidin-3-O-galactoside were more stable with CP. In conclusion, the combination of AME+CP increased bioavailability by reducing oxidative degradation of phenolic compounds, demonstrating the effectiveness of cold plasma technology in improving phenolic stability in functional foods.

Keywords: Cold plasma, wheat grass, *Aronia melanocarpa* extract, protein-phenolic interaction.

1 Introduction

Aronia melanocarpa anthocyanins are compounds that undergo rapid degradation, particularly at intestinal pH, and only 15–60% remain accessible after digestion (Pérez-Vicente et al., 2020; Kumkum et al., 2024). Therefore, increasing the stability of anthocyanins using cold plasma (CP) is a critical need for functional food develop-

ment. In this context, cold plasma is considered an innovative process in this project due to its potential to modify phenolic structures, increase oxidative stability, and improve the antioxidant capacity of extracts.

Wheatgrass (WG) is rich in chlorophyll, phenolic acids, flavonoids, and vitamins, and its antioxidant, anti-inflammatory, and anti-cancer effects have been demonstrated in the literature (Chauhan, 2014; Kumar et al., 2021). Furthermore, thanks to its protein components, it can form complexes with anthocyanins and act as a protective matrix during digestive conditions. This project will investigate the interaction of cold plasma-treated aronia phenolics with wheatgrass protein to reveal changes in phenolic compounds. The interaction of aronia phenolics with wheatgrass protein and the effect of cold plasma treatment on this system have not yet been investigated in the existing literature. Therefore, this study offers a unique approach regarding phenolic-protein interactions and the contribution of plasma technology to functional food development. Although phenolic compounds have high antioxidant activity, their bioaccessibility is limited due to degradation during the digestive process (Günel-Koroğlu et al., 2023; Dai et al., 2020). Plant proteins play an important role in functional food design by acting as natural carriers that can protect phenolics from oxidative degradation.

2 Material Methods

2.1 Cold Plasma Application

The low-pressure CP system operated under atmospheric conditions and utilized a capacitive discharge mechanism with eight electrodes arranged in a circular chamber. Each electrode measured 100 mm in length. The system incorporated a rotary chamber that functioned at 10 rpm, with a frequency of 13.56 Hz and a maximum power output of 100 W. The plasma chamber was constructed from borosilicate glass. Inside the chamber, four rotary columns facilitated the rotation of sample vials (Femto type; Diener Electronic, Ebhausen, Germany). A rotary pump was employed for gas discharge, with an intake capacity of 6 m³/h (Pfeiffer, Asslar, Germany). AME+WG (100:100, 100:50, 100:25) samples were accurately weighed and placed into 200 mL square-shaped bottles, with 5 g of each sample carefully positioned. Plasma treatment was performed at room temperature under rotational conditions, using a power setting of 80 W and a pressure of 0.60 mbar for durations of 5, 10, and 15 min.

2.2 Spectrophotometric Assays

Total phenolic content (TPC) analysis was performed according to the method reported by Singleton and Rossi (1965). Folin–Ciocalteu reagent (112 µL of 0.2 N) was added to each 15 µL sample, followed by 112 µL of 6% (w/v) sodium carbonate (Na₂CO₃) solution. The mixtures were incubated in the dark for 1 h. Absorbance values were measured with a spectrophotometer (BioTek Instruments, Winooski, USA) at a wavelength of 765 nm. The calibration curve was established using gallic acid standard ($R^2 = 0.9904$, $y = 3.1324x - 0.1109$) and the results were expressed as milligram gallic acid equivalents (mg GAE) per 100 mL.

Copper (II) ion reduction capacity-based antioxidant capacity analysis (CUPRAC) was applied according to the method developed by Apak et al. (2004). Each 7 μL sample was mixed with 70 μL of 0.01 mM copper (II) chloride solution, 70 μL of 0.0075 M neocuprine solution, 70 μL of ammonium acetate buffer adjusted to pH 7.0 and 70 μL of distilled water, respectively. The prepared mixtures were incubated in the dark for 30 min. Absorbance values were measured at 450 nm and evaluated using the calibration curve ($R^2 = 0.9905$, $y = 2.3423x - 0.0761$) created with Trolox standard. The results were reported as milligram Trolox equivalents (mg TE/100 mL).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity was determined based on the method of Rai et al. (2006). Each 10 μL of sample was mixed with 200 μL of DPPH solution prepared with 0.01 mM DPPH solution in 75% methanol. The mixtures were incubated in the dark for 30 min. Absorbance measurements were carried out at 517 nm wavelength and evaluations were made on the calibration curve prepared with Trolox standard ($R^2 = 0.9913$, $y = 1.1354x - 0.0074$). Results were expressed as milligram Trolox equivalents (mg TE/100 mL).

2.3 FTIR Analysis

The secondary structure of the wheat grass, was examined with an IRTracer-100 FTIR spectrometer (Shimadzu, Kyoto, Japan). This analysis was conducted by averaging 10 scans and an average of 10 scans at a resolution of 4 cm^{-1} , covering a wavelength range from 400 cm^{-1} to 4000 cm^{-1} , as He et al. (2022) described.

3 Result and Discussion

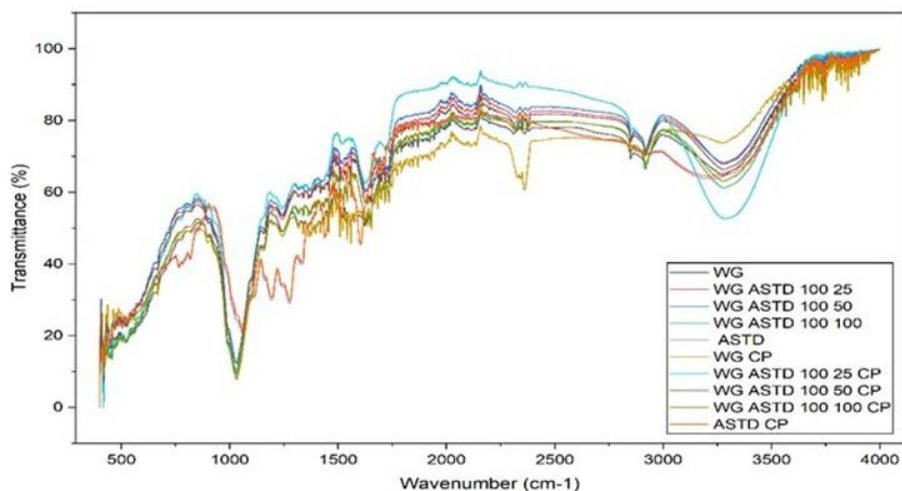


Fig. 1. FT-IR Analysis of samples

As seen in Figure 1, the spectra show the structural differences between wheatgrass (WG), aronia phenolics (ASTD) and cold plasma (CP)-treated samples. Changes in the broad O–H/N–H bands in the 3000–3600 cm^{-1} region indicate that the hydrogen bond density increases with the binding of phenolics to the protein; shifts in the amide I–II regions (1700–1500 cm^{-1}) indicate that the protein secondary structure is modified by both phenolic interaction and plasma treatment. While the decreases in the peaks in the 1400–1600 cm^{-1} region, where aromatic structures are located, support those phenolic compounds are adsorbed onto the protein, the fact that these changes are more pronounced in CP-treated samples indicates that plasma strengthens the binding. The intense variations in the fingerprint region (400–1500 cm^{-1}) reveal that cold plasma causes additional modifications on both polysaccharide and carbonyl structures; Overall, all findings indicate that complexation occurs between aronia phenolics and wheatgrass proteins and that plasma treatment enhances this interaction.

Table 1. Phenolic and antioxidants values of each samples

| Sample | Description | TPC (mg GAE/g) | DPPH ($\mu\text{mol TE/g}$) | CUPRAC ($\mu\text{mol TE/g}$) |
|---------|-------------|-------------------------------------|------------------------------------|---------------------------------------|
| WG | | 740.59 \pm 39.37 ^c | 2197.41 \pm 134.88 ^f | 2866.99 \pm 269.53 ^b |
| WG+ASTD | 100:25 | 2516.54 \pm 159.32 ^c | 9195.66 \pm 793.29 ^b | 8140.00 \pm 586.35 ^b |
| WG+ASTD | 100:50 | 1892.34 \pm 165.22 ^c | 3211.78 \pm 10.76 ^{ef} | 5222.06 \pm 396.23 ^b |
| WG+ASTD | 100:100 | 1482.48 \pm 49.09 ^c | 4304.62 \pm 240.73 ^{de} | 5196.69 \pm 267.67 ^b |
| ASTD | | 46447.57 \pm 4946.97 ^b | 43740.79 \pm 724.34 ^a | 151227.08 \pm 13705.13 ^a |
| WG+CP | | 750.53 \pm 58.14 ^c | 3637.86 \pm 781.24 ^c | 4234.38 \pm 827.20 ^b |
| WG+ASTD | 100:25+CP | 2068.52 \pm 77.98 ^c | 6706.48 \pm 288.84 ^c | 6433.82 \pm 320.17 ^b |
| WG+ASTD | 100:50+CP | 3205.57 \pm 185.12 ^c | 6332.23 \pm 152.88 ^c | 8595.59 \pm 346.14 ^b |
| WG+ASTD | 100:100+CP | 1813.83 \pm 79.61 ^c | 5451.38 \pm 639.26 ^{cd} | 5709.56 \pm 346.47 ^b |
| ASTD+CP | | 64938.40 \pm 7908.24 ^a | 42626.34 \pm 290.30 ^a | 158421.75 \pm 17706.0 ^a |

As shown in Table 1, the effects of wheat sprout protein (WG), aronia extract (ASTD), and cold plasma (CP) treatments on total phenolic content (TPC), radical scavenging capacity (DPPH), and reducing power (CUPRAC) were evaluated in this study. ANOVA results showed highly significant differences between the groups in all parameters ($p < 0.001$). In addition, the high calculated effect sizes ($\eta^2 > 0.98$) reveal that these treatments have a strong effect on antioxidant capacity (Amiri et al., 2025). According to the Scheffé test results, the WG control group had the lowest phenolic content and antioxidant capacity. ASTD supplementation provided a significant increase in phenolic content and antioxidant capacity, and it was observed that ASTD, especially applied at high rates, provided statistically significant improvements in DPPH and CUPRAC values.

The WG+CP and WG+ASTD+CP groups showed higher TPC, DPPH, and CUPRAC values compared to the groups without CP. The highest values were obtained in the ASTD+CP group. This result indicates that CP reduces oxidative losses by increasing the structural stability of phenolic compounds (Porto et al., 2024). The findings are consistent with studies in the literature indicating that CP increases antioxidant capacity by preventing the oxidative degradation of phenolic compounds (Amiri et al., 2025; Porto et al., 2024). In addition, aronia extract is known to enhance reducing power and radical scavenging activity due to its polyphenolic structure. The contribution of ASTD was clearly observed in this study, and the addition of CP demonstrated that this effect was further enhanced. In conclusion, CP technology can be considered an effective method for increasing the bioavailability of phenolic compounds in functional foods and enhancing the antioxidant capacity of the product. The highest performance of the ASTD+CP combination, in particular, demonstrates the promising future potential of this technology for preserving phenolic compounds in food matrices and enhancing their functional properties.

4 Conclusion

This study demonstrates that *Aronia melanocarpa* phenolics form strong interactions with wheatgrass protein, and cold plasma treatment significantly strengthens these interactions both structurally and functionally. FTIR results revealed that phenolic compounds bind to the protein, forming a more stable complex, and that cold plasma enhances this binding, partially rearranging the protein structures. Antioxidant analyses confirmed the functional correlates of these structural changes; the addition of aronia phenolics significantly increased antioxidant capacity, while cold plasma treatment preserved phenolic compounds and further enhanced bioactivity. The highest phenolic content and antioxidant capacity were observed in plasma-treated aronia samples, demonstrating that cold plasma is an effective technology for enhancing phenolic stability. Overall, the study demonstrates that CP enhancement of plant protein-phenolic interactions offers a powerful strategy for developing phenolic-rich, more stable, and highly bioavailable functional food ingredients.

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