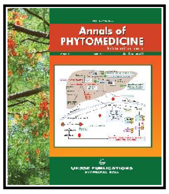
Annals of Phytomedicine 12(2): 937-942, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.2.111

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Enhancing walnut (*Juglans regia* L.) safety and quality: Unveiling the potential of radio frequency cold plasma treatment

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Article Info

Article history
Received 20 August 2023
Revised 7 October 2023
Accepted 8 October 2023
Published Online 30 December 2023

Keywords
Ecofriendly food processing
Microbial safety
Non-thermal walnut processing
Sustainable food processing

Abstract

In the context of food safety, this study explored the application of radio frequency cold plasma (RF-CP) technology to enhance the microbial safety of dried walnuts. Four different gas combinations, including nitrogen (N2), argon (Ar), N_2+O_2, and Ar+O_2, were employed for RF-CP treatment. Various power levels (ranging from 60 to 210 W in 10% increments) and treatment durations (5, 10, 15, and 20 min) were tested. Results demonstrated a direct relationship between plasma power and treatment duration, leading to increased microbial reduction. The most significant reductions, exceeding 99%, were achieved at 180 W for 20 min using N_2 (70%) + O_2 (30%) gas (OW-1) and 210 watts for 10 min with Ar (70%) + O_2 (30%) gas (OW-2). Sensory analysis showed acceptable scores for plasma-treated walnuts. No significant differences in nutritional composition were observed, except for decreased moisture content and increased carbohydrate content with higher power and longer RF-CP exposure. This study highlights the potential of RF-CP cold plasma as an eco-friendly method for effectively decontaminating walnuts and similar high-value foods.

1. Introduction

Walnuts (*Juglans regia* L.) are esteemed for their remarkable nutritional profile, characterized by high levels of essential fatty acids, vitamins, minerals, and antioxidants that confer numerous health benefits (Hussain *et al.,* 2021). Despite their nutritional prowess, walnuts are not impervious to microbial contamination, which can pose significant challenges to their safety, shelf-life, and consumer health (Ahangari *et al.,* 2021). Conventional decontamination methods encompassing physical, chemical, and biological approaches have historically been employed to mitigate the presence of deleterious microorganisms and enzymatic catalysts in nuts (Misra *et al.,* 2019; Torun *et al.,* 2018). Nonetheless, the application of these traditional methods may inadvertently result in the diminishment or degradation of thermosensitive nutritional constituents and biologically active compounds present in these nutritionally valuable nuts (Atungulu and Pan, 2012). In the pursuit of safer and less extensively processed food choices, innovative technologies, particularly non-thermal processing methods, have garnered significant attention. These methods have risen to prominence due to their remarkable capability to maintain food quality while efficiently deactivating pathogenic microorganisms. This dual benefit makes them a promising solution for producing safer and minimally processed food options, aligning with the contemporary demand for enhanced food safety and quality (Chacha *et al.,* 2021; Dasan *et al.,* 2016).

One such technology that has garnered considerable attention in recent years is radio frequency cold plasma (RF-CP) treatment. RF-CP treatment combines the power of electromagnetic energy and cold plasma to achieve microbial decontamination (Singh *et al.,* 2019). Radio frequency cold plasma technology is a scientifically and practically significant innovation that addresses several critical challenges facing the food industry. Its primary contribution lies in ensuring microbial safety, effectively deactivating pathogenic microorganisms like bacteria, viruses, and moulds, all without the need for elevated temperatures or chemical interventions (Obileke *et al.,* 2022). Beyond safety, RF-CP plays a pivotal role in extending the shelf-life of food products by significantly reducing the microbial load, thereby reducing food waste and offering economic and environmental benefits. What sets RF-CP apart is its unique capacity to achieve microbial decontamination while preserving the sensory attributes, nutritional integrity, and textural properties of food, a crucial consideration in meeting consumer preferences for fresh and minimally processed foods (Allai *et al.,* 2022). Furthermore, RF-CP's energy efficiency, reduced chemical usage, and lower environmental impact in comparison to traditional thermal methods align perfectly with sustainability objectives and regulatory requirements (Jiang *et al.,* 2022). Its versatility, adaptability, and compliance make it a powerful and scientifically significant tool for the food industry. The technology offers a promising avenue for addressing the food industry's imperative for enhanced safety measures without compromising product quality. To maximize its potential, meticulous

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optimization of key plasma parameters, including considerations such as feed gases, voltage, and exposure duration, is essential (Khodabandeh *et al.,* 2023). In sum, RF-CP technology not only addresses multifaceted challenges in food safety, quality, and sustainability but also underscores its importance as a transformative solution for the food industry. This technology offers a viable avenue for addressing the food industry's imperative for enhanced safety measures without the trade-off of compromised product quality (Khodabandeh *et al.,* 2023).

Cold plasma, a unique state of matter that consists of highly reactive ions, electrons, and neutral species, has gained prominence for its ability to effectively inactivate a wide range of microorganisms while operating at relatively low temperatures (Mandal *et al.,* 2018). As consumer preferences shift towards minimally processed foods with extended shelf-lives, there is a growing demand for innovative techniques that can mitigate microbial risks without compromising the sensory attributes and nutritional integrity of products (Thakur *et al.,* 2020; Bansal *et al.,* 2014). This shift has underscored the significance of technologies like RF-CP treatment in revolutionizing the food industry. RF-CP treatment holds the potential to provide an elegant solution by simultaneously tackling microbial contaminants and preserving the inherent qualities that make walnuts a popular dietary choice (Ahangari *et al.,* 2021).

In the current investigation, the primary objective is to subject walnut kernels to RF-CP treatment utilizing nitrogen, argon, and oxygen gases, both individually and in various gas mixtures with oxygen (ranging from 5% to 50% oxygen content). The RF-CP treatment was applied at six different power levels, incrementing from 60 to 210 W in 10% intervals, relative to the instrument's total power capacity of 300 W. Additionally, four distinct treatment durations (5, 10, 15, and 20 min) were employed. The processed walnut samples underwent comprehensive analyses encompassing proximate composition, microbial load reduction, sensory evaluation. This multifaceted investigation is aimed at discerning the optimal gas combination and treatment parameters that facilitate effective decontamination while preserving the organoleptic and nutritional qualities of walnuts. The findings of this study are anticipated to significantly contribute to the existing body of knowledge on the application of cold plasma technology in the processing and decontamination of walnut.

2. Materials and Methods

2.1 Sample collection and preparation

Fresh walnuts (*Juglans regia* L.) were collected from local market of Varanasi and transported to the laboratory in sanitized HDPE bags. They were kept within a dry environment and maintained at a temperature of 4 ± 1°C. Protection from direct sunlight was diligently ensured during their storage, and this arrangement was upheld until they were ready for use in subsequent experiments. The walnuts were carefully sorted to eliminate any visibly damaged or spoiled ones. Analytical-grade chemicals procured from Sigma Aldrich (Mumbai, India) were utilised exclusively for this research. The research experiments were carried out in CSIR-IMtech and CSIR-CSIO Chandigarh, India.

2.2 Radio frequency cold plasma treatment

The experimental setup involved the utilization of a customized computer-controlled plasma treatment apparatus designed for scientific investigations (PICO, manufactured by M/s Diener electronic GmbH, Germany. This plasma system enclosure was equipped with an Oerlikon rotary vane pump (model D16BCS), a 13.56 MHz RF Generator (0-300 W), and a rotating glass vessel that completed five revolutions per minute. Within each cycle, precisely 200 g of walnut were meticulously placed inside the rotating cylinder of the glass container. The chamber exhibited dimensions of 160 mm in both length and width, with a depth of 325 mm. The power was supplied to the electrode axially positioned within the glass container, driven by a radio frequency (RF) signal operating at 13.56 MHz *via* an automatically adjusting matching network. Notably, the process gas could ingress through the porous cap situated at the tip of the powered electrode.

2.3 Microbial load determination

Representative samples from both control and experimental groups were aseptically collected and analysed for initial microbial load The processed walnut underwent pulverization and were subsequently combined with a sterile sodium chloride (NaCl) solution. Subsequently, 100 µl of the resulting mixture was evenly dispersed onto nutrient agar and potato dextrose agar (PDA) plates for the purpose of enumerating total plate count and yeast and mold colonies, respectively. Preceding the colony enumeration process, the agar plates were subjected to incubation within a thermostatic chamber maintained at 37°C for a specified duration of 24 h to facilitate optimal growth conditions. In contrast, the PDA plates were subjected to incubation at a controlled temperature of 25°C, extending over a period of 5 days (Rodriguez *et al.,* 2017). The microbial load was expressed as colony-forming units (CFU) per gram of walnuts and the reduction in microbial load was calculated as a percentage by comparing the post-treatment count to initial count.

2.4 Proximate analysis

The analysis of moisture, carbohydrate, protein, fat, and ash content in both control walnuts and cold plasma-treated walnuts was conducted in accordance with the procedures specified by the Association of Official Analytical Chemists (AOAC, 2005). The protocol utilized for this analysis was adapted with slight modifications from the methodology described by Jyoti *et al.* (2022).

2.5 Sensory evaluation

Sensory assessment of both control and cold plasma-treated walnut was conducted following a modified version of the nine-point hedonic rating scale method as mentioned by Chuwa *et al.* (2022). The evaluation of sensory attributes was conducted using a 9-Point Hedonic rating scale to assign scores (Gautam *et al.,* 2020; Verma *et al.,* 2021). In the sensory evaluation of both the untreated (control) and cold plasma-treated walnut samples, multiple sensory attributes were assessed, encompassing considerations of colour, appearance, flavour, odour, taste, texture, and mouthfeel characteristics. A Hedonic scale ranging from 1 to 9 was employed for this purpose, with the lowest rating (1), indicating a strong disliking of the attribute, and the highest rating (9), indicating a strong liking (Gautam *et al.,* 2020). The collective score, *i.e.*, the overall acceptability of these assessed qualities was then utilized to calculate an overall acceptability score, represented through Figure 2. This sensory evaluation enlisted the expertise of 15 semi-trained panellists, who applied a 9-Point Hedonic scale to provide their assessments. They were duly informed about the product composition and its non-toxic nature to ensure a comprehensive understanding. The panellists were instructed to cleanse their palates before and after sampling each individual sample and were tasked with evaluating various sensory attributes, including colour, texture, flavour and overall acceptability. The walnut samples were presented in square glass containers alongside plain water for palate cleansing, with the sensory assessment conducted at room temperature and the scores were statistically analysed.

2.6 Statistical analysis

In this study, a standardized feed gas composition, power, treatment duration was established through a series of controlled experiments. The experiments were meticulously executed in triplicate to ensure the reliability of the results. Subsequently, data analysis was conducted utilizing Minitab 16 software. To ascertain the impact of the radio-frequency cold plasma treatment, a one-way analysis of variance (ANOVA) was employed. The distinctions among the means were further elucidated through Tukey's test, employing a significance level of ($p<0.05$).

3. Results

3.1 Optimal time and dosage selection for effective treatment

The study aims to optimize cold plasma treatment for microbial inactivation in walnuts with minimal sensory impact. Various gas combinations, powers, and time intervals were evaluated. Nitrogen, argon, and oxygen gases were the primary plasma contributors. Nitrogen and argon were tested alone and in combinations with oxygen (5% to 50%). Powers (60 to 210 W) and time segments (5 to 20 min) were selected. Powers below 60 W were ineffective, and a 10% power difference was chosen for optimal results. Time intervals (≤ 5 to < 20 min) were determined to balance microbial reduction and sensory impact. Nitrogen and argon with 30% oxygen proved effective for maximum microbial eradication with minimal sensory disruption.

Although, all the gases tested in this study were effective against microbial inactivation yet the higher energy consumption led to sensory disruption. The results of this study suggest that nitrogen and argon gases, combined with 30% oxygen, are the optimal gas mixture for cold plasma treatment of walnuts. This mixture was found to be effective in microbial inactivation while minimising sensory disruption.

3.2 Microbial decontamination

Figure 1 presents a comprehensive analysis of the impact of cold plasma treatment on microbial populations, specifically the total plate count, yeast count, and mold count, employing two different gas mixtures: N_2 (70%) + O_2 (30%) and Ar (70%) + O_2 (30%), denoted as OW-1 and OW-2, respectively. Our investigation revealed a significant reduction ($p<0.05$) in total plate count, yeast and mold populations with increasing plasma power and exposure duration. As depicted in Figure 2, the cumulative microbial load in walnut samples subjected to cold plasma treatment exhibited a consistent decrease relative to the control group as treatment intensity and duration were augmented. Initially, at lower power levels and shorter exposure durations, the microbial reduction rate was observed to be relatively sluggish. However, a discernible trend emerged as both plasma power and exposure time increased, resulting in a gradual escalation in the rate of microbial reduction. Notably, the most substantial reduction, exceeding 99%, in total plate count, yeast count, and mold count was achieved under specific plasma conditions: 180 W of power for 20 min utilizing the N_2 (70%) + O_2 (30%) gas mixture (OW-1) and 210 watts for 10 min employing the Ar (70%) + O_2 (30%) gas mixture (OW-2). Importantly, these conditions were found to effectively decontaminate walnuts while preserving their sensory attributes. The outcomes of this investigation highlight the superior efficacy of the N_2 and O_2 gas mixture in microbial decontamination, accomplishing the desired microbial reduction at lower power levels.

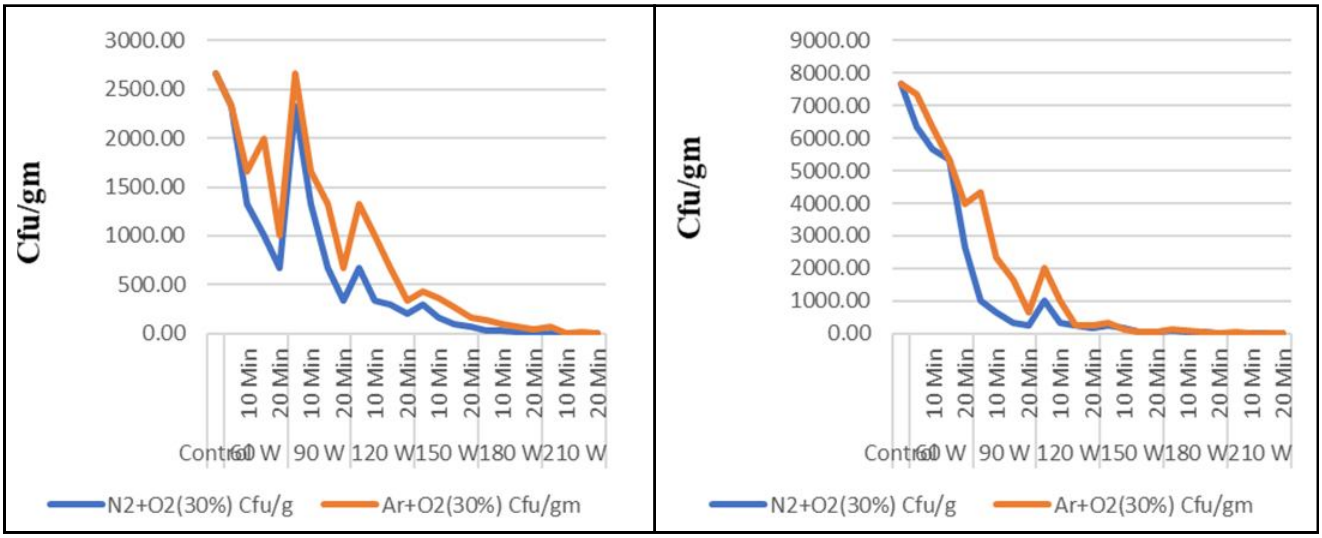

Figure 1: Decontamination efficiency of radio frequency cold plasma.

3.3 Effect of RF-CP treatment on proximate composition of walnut

The impact of RF-CP treatment on the moisture, water activity (aw), fat, protein, carbohydrate, and ash content of walnuts was assessed under two distinct treatment conditions, referred to as OW-1 and OW-2, and compared to untreated control walnuts. The results are summarized in Table 1. Initially, the moisture content of the walnuts was 4.08 ± 0.50%, which was significantly altered by the RF-CP treatment, resulting in a decrease to 3.13 ± 0.24% (OW-1) and 2.84 ± 0.10% (OW-2), respectively. Regarding water activity, our analysis revealed that cold plasma exposure had no statistically significant effect when compared to unprocessed walnuts. However, it is worth noting that walnuts treated under OW-2 conditions exhibited a slightly lower water activity (aw= 0.38 ± 0.10) than those treated under OW-1 conditions (aw= 0.41 ± 0.14) and untreated walnuts (aw = 0.51 ± 0.12), respectively. The fat content of walnuts demonstrated minor variations, with walnuts treated under OW-2 conditions showing a slightly higher fat content (65.25 ± 0.45) than those under OW-1 conditions (64.79 ± 0.75) and the control group (65.15 ± 0.35), respectively. Nevertheless, these differences were not statistically significant, indicating comparable fat content among the groups. The protein composition of cold plasma-treated walnuts did not exhibit any statistically significant differences when compared to the control group. However, a slight increase was observed, with protein content rising from 19.37 ± 1.24% in untreated walnuts to 20.09 ± 1.14% (OW-1) and 20.34 ± 1.02% (OW-2) following cold plasma treatment. Perhaps the most intriguing finding in this study was the significant increase in carbohydrate content in cold plasma-treated walnuts. Specifically, carbohydrate content increased from 9.35 ± 0.7% in untreated walnuts to 11.33 ± 1.0% (OW-1) and further to 12.50 ± 1.45% (OW-2) after cold plasma treatment. These results provide valuable insights into the compositional changes induced by cold plasma treatment in walnuts. Notably, no significant alterations were observed in the ash content across all experimental groups.

Table 1: Proximate composition of control and cold plasma treated walnut under optimised condition

Parameters	Control	OW-1 ($N_2 + O_2$)	OW-2 ($Ar + O_2$)
Protein (g)	19.37 ± 1.24^a	20.09 ± 1.14^a	20.34 ± 1.02^a
Carbohydrate (g)	9.35 ± 0.7^b	11.33 ± 1.0^c	12.50 ± 1.45^a
Moisture (%)	4.08 ± 0.50^b	3.13 ± 0.24^c	2.84 ± 0.10^c
Water activity (a_W)	0.51 ± 0.12^b	0.41 ± 0.14^b	0.38 ± 0.10^b
Ash (%)	2.05 ± 0.08^a	2.02 ± 0.02^a	2.03 ± 0.05^a
Fat (g)	65.15 ± 0.35^a	64.79 ± 0.75^a	65.25 ± 0.45^a

Note: Value reported as Mean ± SD of three replications. Means followed by different superscripts within a row for same parameter are significantly different (*$p<0.05$*) by DMRT.# OW= Optimised walnut sample Top of Form.

3.4 Sensory analysis

The results of the sensory evaluation, as illustrated in Figure 2, demonstrate a substantial impact of plasma parameters on the sensory attributes of the cold plasma treated walnuts. Notably, the plasma-treated group exhibited sensory scores that were lower in comparison to the control group (8.5 ± 0.25). However, when subjected to the OW-1 and OW-2 conditions, the sensory scores consistently surpassed sensory score of 6, specifically recording values of 6.8 ± 0.15 and 6.5 ± 0.13, respectively, across all assessed parameters. Interestingly, as the plasma power levels were progressively increased, a discernible decline in sensory scores was observed, ultimately reaching 5.8 ± 0.10 and 6.1 ± 0.30 at the highest power settings under the OW-1 and OW-2 plasma conditions, respectively. These findings underscore the intricate relationship between plasma treatment parameters and the sensory characteristics of treated walnuts, offering insights into the delicate balance required for achieving microbial decontamination while maintaining sensory quality.

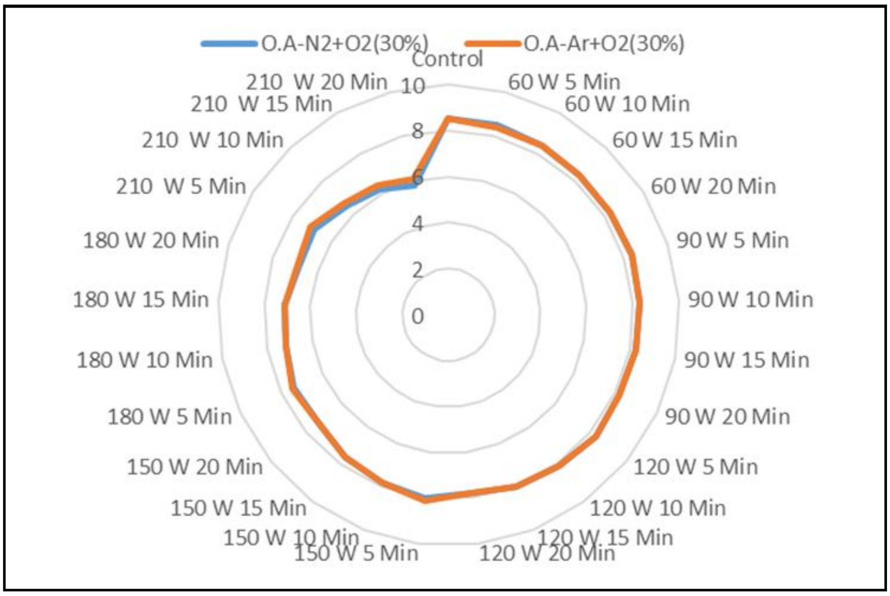

Figure 2: Sensory score of cold plasma treated walnut under OW-1 and OW-2.

4. Discussion

The results of this study provide valuable insights into the effectiveness of cold plasma treatment for microbial decontamination in walnuts. The reduction in total plate count, yeast count, and mold count under optimal conditions OW-1 (180 W for 20 min with N_2-70% + O_2-30%) and OW-2 (210 W for 10 min with Ar-70% + O_2-30%), exceeding 99%, highlights the potential of this technology in enhancing nuts safety. The proposed mechanisms involving the generation of reactive entities and electric fields shed light on why cold plasma is so effective in microbial inactivation (Gavahian *et al.,* 2018; Sohbatzadeh *et al.,* 2016). These results align with previous research such as Devi *et al.* (2017); Thirumdas *et al.* (2015); Makari *et al.* (2021) and Ahangiri *et al.* (2021) although the differences in outcomes across studies emphasize the importance of various factors, such as gas mixtures and processing conditions. The impact of cold plasma treatment on the proximate composition of walnuts revealed noteworthy changes in moisture, fat, protein, and carbohydrate content. These changes, particularly the increase in carbohydrate content, provide valuable insights into the compositional alterations induced by cold plasma treatment and the results except the rise of carbohydrate content were in line with the findings of Sohbatezadeh *et al.* (2016); Selcuk *et al.* (2008); Bahrami *et al.* (2016); Choi *et al.* (2016). The possible causes for an increase in carbohydrate content after cold plasma treatment can include starch gelatinization, the Maillard reaction, formation of oligosaccharides, cross-linking reactions, adsorption of atmospheric gases, and changes in sample moisture content. These factors can lead to alterations in the carbohydrate composition and content of the treated material (Warne *et al.,* 2021). Additionally, the maintenance of sensory attributes within acceptable limits under OW-1 and OW-2 conditions highlights the potential for microbial decontamination without compromising overall product quality. It is important to note that water activity remained relatively stable, which is crucial for inhibiting microbial growth and enhancing shelf stability. However, further research could delve into the potential impact of other sensory attributes, such as taste and aroma, to provide a more comprehensive understanding of the technology's effect on sensory quality.

5. Conclusion

The research findings strongly indicate that RF-CP treatments under OW-1 and OW-2 conditions effectively reduced total plate count and yeast and mold count compared to untreated samples. Optimal conditions for microbial decontamination were found at 180 W for 20 min (N_2-70%+O_2-30%, *i.e.*, OW-1) and 210 W for 10 min (Ar-70% + O_2-30%, *i.e.*, OW-2). These parameters achieved more than 99% microbial reduction without significant alterations in nutritional and sensory attributes, although there was a slight decrease in moisture content and an unexpected increase in carbohydrate content. The results indicate that cold plasma technology holds promise as an innovative method to effectively reduce microbial contamination in walnut while preserving their nutritional integrity. These findings may contribute to the development of novel decontamination techniques for dried foods and other valuable food products.

Acknowledgements

The authors gratefully acknowledge to CSIR-IMtech for providing the microbial studies facility and CSIR-CSIO Chandigarh for providing the RF-CP facility. The authors are thankful to Dr. Suresh Korpole, Dr. K. Murthi (CSIR-Imtech) and Ms. Harjit Kaur (CSIR-CSIO for their valuable guidance. Authors would also like to acknowledge to institution of Eminence (IoE) scheme (Dev. Scheme No. 6031 and PFMS scheme No. 3254), BHU-Varanasi (UP) India for the financial support.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Rajeev Ranjan, Anil Kumar Chauhan, Dhan Prakash, Rajesh Singh, Shubhendra Singh and Pallawi Joshi (2023). Enhancing walnut (*Juglans regia* L.) safety and quality: Unveiling the potential of radio frequency cold plasma treatment. Ann. Phytomed., 12(2):937-942. http://dx.doi.org/10.54085/ap.2023.12.2.111.**