



Original research

The Effects of Cold Plasma on Physicochemical and Microbial Properties of Mint and Echium

Arash Jazayeri¹, Soheila Abdi^{2*}¹. Department of Food Science and Technology, Safadasht Branch, Islamic Azad University, Tehran, Iran.². Department of Physics, Safadasht Branch, Islamic Azad University, Tehran, Iran.

A B S T R A C T

Two important medicinal plants used in traditional Iranian medicine are Echium and Mint. Due to the importance of echium and mint in medicine, the use of appropriate disinfection methods without affecting their quality needs more attention. A new method in decontamination of spices is the use of cold plasma technology. This study aimed to evaluate cold plasma effects on microbial and physicochemical properties of Echium and Mint compared to the heating method. One gram of Echium and Mint leaf powder was spread on the glass slide as a single layer and was exposed to the plasma for 30 min. Phenolic compounds and color changes of Mint and Echium under the exposure of plasma and heat were measured. The results showed that plasma had no significant effect on phenolic compounds and color changes ($p \geq 0.05$) while the heating method had a significant effect on phenolic compounds and color changes ($p < 0.05$). Therefore, plasma can be a suitable alternative to the heating method in the decontamination of spices without affecting the nutraceutical and quality.

Keywords: Echium, Mint, Cold Plasma, Phenolic Compound, Microorganism

Received 6 April 2024; 2024; Accepted 23 Aug. 2024

This is an open-access article distributed under the terms of the Creative Commons Attribution- 4.0 International License which permits Share, copy and redistribution of the material in any medium or format or adapt, remix, transform, and build upon the material for any purpose, even commercially.

1. Introduction

One of the highest categories of therapies that have attracted the attention of scientists is analgesics (Elisabetsky and Castilhos, 1990). Due to the wide range of side effects of analgesics, study on new and better compounds is needed (Katzung, 2001). Various herbs are used in traditional medicine as analgesics (Mandegary et al., 2004, Mills and Bone, 2000). Two important medicinal plants used in traditional Iranian medicine are *Echium amoenum* and Mint (Hooper and Field, 1937). *Echium amoenum* petals have long been used as an analgesic, anti-inflammatory, and anti-anxiety drug in Iran (Hooper and Field, 1937). Mint has antimicrobial and antioxidant properties and is used as an anti-microbial and anti-inflammatory, stomach tonic, anti-cough, anti-convulsant, astringent, stimulant, pain reliever drug in traditional medicine (Hooper and Field, 1937, Hussain et al., 2010). *Echium amoenum* and Mint may be infected by pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus*

scereus, and *Salmonella* during harvesting, drying, transporting and packing process (Alavi et al., 2017, Ernst, 2002, Khodadadi et al., 2012). Infection of Echium and mint with bacteria and molds limits their use in medicine, so it is essential to use decontamination methods without changing their quality. The methods commonly used to decontaminate plants are time-consuming and harmful. Recently, cold plasma technology has been used as an alternative to the process of sterilizing and disinfecting herbs, spices, and seeds (SádECKá, 2007, Sospedra et al., 2010). UV photons, electrons, free radicals, positive and negative ions, atoms, and excited molecules in cold plasma can inactivate bacteria in plants without thermal effects (Bhatt et al., 2018). One of the important elements in plasma is ionization. The interaction of ions with cells can induce antimicrobial effects of plasma. Reactive species in plasma have oxidative effects on the cell surface of microbes. An active species in plasma destroys DNA and subsequently inactivates bacteria (Abdi et al., 2016).

*Corresponding author: E-Mail Address: abdi.soheila@gmail.com

Inactivation of air bacterial by cold plasma in spores and vegetative cells takes less time (Philip et al., 2002). Studies have shown that cold plasma kills bacteria in red pepper powder (Abdi et al., 2019).

Studies have shown that cold plasma kills bacteria in red pepper powder (Abdi et al., 2019). The effect of cold plasma on lettuce showed that the number of *L. monocytogenes* decreased by 3 logarithmic cycles in three minutes and 5 logarithmic cycles in five minutes (Niemira and Sites, 2008). Surface disinfection of spices and dried plants, such as Echium and Mint by plasma can be a new method of decontamination without unwanted quality damage. In this experimental research, the comparison between two microbial decontamination methods (cold plasma and heating process) on the phenolic compounds and the quality of Echium and mint was studied.

2. Materials and methods

FE-DBD plasma source

The instrument used to create a low-temperature atmospheric-pressure cold plasma was a radiofrequency power supply and an electrical discharge chamber which was described in our previous report. One gram of Echium and mint leaf powders were spread on the glass slide as a single layer and exposed to the plasma for 30 min. The method of plasma exposure and evaluation of colonies are explained in the previous report (Abdi et al., 2019).

Heating method

To decontaminate Echium and mint leaf, a bain-marie device was used. The samples were heated at 70°C for 15 min.

Total count of microorganisms

The total count of microorganisms was investigated and counted according to the national standard number 5272 in the culture medium of count agar plate and temperature of 30° for 72 hs in 5 dilutions. In order to count the colonies, the following formula was used.

$$N = \frac{\sum C}{\{(n1 \times 1) + (n2 \times 0.1)\} d}$$

That in which N is the colonies number per mL of the product, $\sum C$ is sum of colonies in all counted plates, n1 is plates number of lower dilution counted, n2 is plates number of higher dilution counted and d is dilution level corresponding to first count.

Determination of phenolic compounds

To measure the phenolic compounds of mint and Echium, Folin-Ciocalteu reagent was used as a reactive material. Reduction of the oxidized hydroxyphenol group by phosphotungstic acid present in the reagent causes the formation of blue color with maximum absorption at 765 nm. Ten minutes after mixing 0.25 mL of samples with Folin-Ciocalteu reagent, 0.375 mL of 20% sodium carbonate was added to it and mixed well. After 2 hours of storage at ambient temperature and away from light, the absorbance was measured at a wavelength of 765 nm. Gallic acid was used as standard and the results were calculated based on mg of Gallic Acid Equivalents (GAE)/kg of dry matter (Feng et al., 2019).

Color changes measurement

The Hunter Lab Chroma Meter CR-400 color measuring system (made in Japan) was used to investigate the color changes of the Echium flower and mint samples. Three factors of color, including lightness (L^*), redness (a^*), and yellowness (b^*), were measured to evaluate color changes in the samples. The range from 0 to 100 (black to white) shows the L^* component, component a^* is expressed from (negative value) to red-purple (positive value), and Component b^* is shown from yellow (positive value) to blue (negative value). The total color difference is calculated from the following formula of $\Delta E_{L^*a^*b^*} = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$.

Statistical analysis

Statistical analysis was carried out using SPSS Statistical Software v. 16.0 (SPSS, Chicago, IL, USA). Comparison of data between the control and exposed samples was done using one-way analysis of variance test. Each experiment was repeated five times. Data were expressed as mean \pm SD (standard deviation). A P-value = 0.05 was considered statistically significant.

Results and Discussion

In this experimental research, the effects of plasma and heat on mint and Echium were investigated and compared. Figure 1 shows that the density of surviving microorganisms in mint and Echium has decreased significantly ($p < 0.05$) under the exposure of FE-DBD plasma in 30 min. One of the factors involved in destroying microorganisms is the electric fields caused by the movement of charged particles in the plasma, these fields cause the outer membrane of the bacterial cell to tear and as a result, destroy it (Jordan et al., 2013). The process of destroying microorganisms by plasma can be done in three steps, first, the genetic material is damaged by ultraviolet radiation. The second stage is described by the absorption of ultraviolet radiation by bacteria and the breaking of the chemical bonds of the wall by UV photon energy. Destruction occurs in the third stage, which depends on various factors, such as the type of microorganism, the type of plasma, and the environment in which the bacteria is located (Salarieh and Dorrani, 2013). Our previous studies showed that microorganisms in red pepper and Nigella Sativa L. were destroyed following exposure to cold plasma (Abdi et al., 2019, Abdi et al., 2020). It has been shown that DC corona discharge plasma can inactivate bacteria (Dobrynin et al., 2011). It has been demonstrated that high-voltage atmospheric cold plasma inactivates *Bacillus atrophos* spores (Patil et al., 2014). Hertwig et al. reported that remote plasma significantly reduced the microbial flora in paprika (Hertwig et al., 2015).

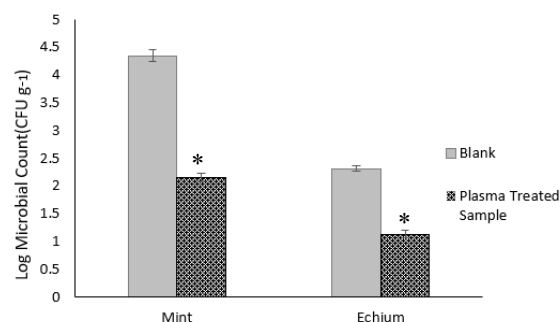


Figure 1. The density of surviving microorganisms in mint and Echium. The values expressed as mean \pm standard derived from separate tests. * $P < 0.05$.

The results show that the amount of phenol in mint and Echium flower does not change significantly under the exposure of plasma, but heat caused a significant decrease in the amount of phenol in both plants (Table 1, 2). The role of phenolic compounds in natural sources is due to their antioxidant activity and preventing the formation of free radicals by donating hydrogen and electrons (Amiri, 2012). Studies have shown that plasma exposure induced phenolic compounds in siriguela juice (Paixão et al., 2019). It has been shown that cold plasma can enhance the phenolic compounds of grape pomace (Bao et al., 2020). It has been found that using a temperature of 70 degrees has caused a significant decrease in phenolic compounds, which is consistent with our results in this study (Pankaj et al., 2018).

Table 1. Phenolic content of Echium under the exposure of cold plasma comparison with heating method.

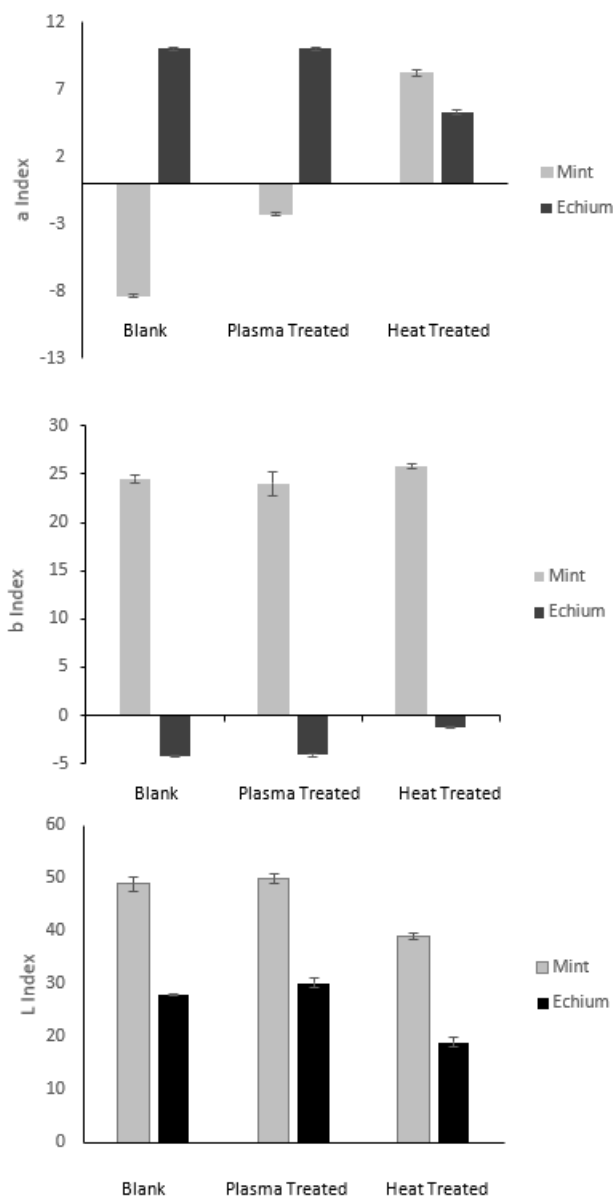
Control	plasma	heat
294.75 \pm 1.06	292.8 \pm 1.13	261.95 \pm 1.76

The values expressed as mean \pm standard derived from separate tests. * $P < 0.05$

Table 2. Phenolic content of mint under the exposure of cold plasma comparison with heating method.

Control	plasma	heat
295.6± 0.84	294.2± 0.28	244.4± 0.98*

The values expressed as mean ± standard derived from separate tests.
* $P < 0.05$

**Figure 2 (2a: Change of parameters a, 2b: Change of parameters b, and 2c: Change of parameters L) in mint and Echium under the exposure of plasma and heat.**

The results of the average of total color changes in the Echium and mint under the influence of heat and plasma are shown in Figure 2 (2a,2b,2c). As can be seen from the results parameters L* and a* had a significant change under the influence of heat ($p < 0.05$). The results show heat increases parameter a* and decreases parameter L*. Parameter a* is the tendency of green color towards red, increasing the value of parameter a* shows that mint green color

has turned towards red under the influence of heat. Parameter L* shows the brightness of the sample. The decrease of parameter L* shows that the mint color has become darker. No significant changes in the color parameters were observed under the influence of plasma. Figure 3 shows that plasma had no significant effect on the color parameters of Echium flowers, but heat decreased parameters a* and L* and increased parameter b*. Studies have shown that the parameters L*, a*, and b* of blueberry color change under exposure to atmospheric cold plasma (Lacombe et al., 2015). Reports have shown that cold atmospheric gas phase plasma changes the color of pomegranate juice (Herceg et al., 2016). It is known that FE-DBD plasma can change the color of red pepper (Abdi et al., 2019).

Conclusion

As a result, the plasma method is a more suitable method than heating in decontamination of mint and Echium, because it did not affect the quality and color of mint and Echium, while the heating method caused a change in the phenolic compounds and color of these spices. Therefore, plasma can be a suitable alternative to the heating method in decontamination of spices without affecting their quality.

References

- Abdi S, Hosseini A, Moslehishad M, Dorrani D. 2019. Decontamination of red pepper using cold atmospheric pressure plasma as alternative technique. *Applied Food Biotechnology*, 6(4):247-54.
- Abdi S, Moslehishad M, Dejam L. 2020. Effect of atmospheric pressure floating-electrode dielectric-barrier discharge (FE-DBD) plasma on microbiological and chemical properties of *Nigella sativa* L. *Journal of Basic Research in Medical Sciences*, 7(1):61-9.
- Alavi I, Zahedi M, Zahedi M, Ghasemi Pirbalouti A, Rahimi E, Momtaz H. 2017. Evaluating the microbial contamination of some Iranian dried medicinal plants and distillates. *International Journal of Epidemiologic Research*, 4(2):118-24.
- Amiri H. 2012. Essential oils composition and antioxidant properties of three thymus species. *Evidence-Based Complementary and Alternative Medicine*, 2012.
- Bao Y, Reddivari L, Huang J-Y. 2020. Enhancement of phenolic compounds extraction from grape pomace by high voltage atmospheric cold plasma. *LWT*, 133:109970.
- Bhatt HK, Prasad R, Joshi D, Sagarika N. 2018. Non-thermal plasma system for decontamination of fruits, vegetables and spices: A review. *International Journal of Communication Systems*, 6(2):619-27.
- Dobrynin D, Friedman G, Fridman A, Starikovskiy A. 2011. Inactivation of bacteria using dc corona discharge: role of ions and humidity. *New journal of physics*, 13(10):103033.
- Elisabetsky E, Castilhos Z. 1990. Plants used as analgesics by Amazonian caboclos as a basis for selecting plants for investigation. *International Journal of Crude Drug Research*, 28(4):309-20.
- Ernst E. 2002. Toxic heavy metals and undeclared drugs in Asian herbal medicines. *Trends in pharmacological sciences*, 23(3):136-9.
- Feng Z, Li Y, Li M, Wang Y, Zhang L, Wan X et al. 2019. Tea aroma formation from six model manufacturing processes. *Food chemistry*, 285:347-54.
- Hertwig C, Reineke K, Ehlbeck J, Erdoğan B, Rauh C, Schlüter O. 2015. Impact of remote plasma treatment on natural microbial load and quality parameters of selected herbs and spices. *Journal of Food Engineering*, 167:12-7.
- Hooper D, Field H. 1937. Useful plants and drugs of Iran and Iraq. Hussain AI, Anwar F, Nigam PS, Ashraf M, Gilani AH. 2010. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. *Journal of the Science of Food and Agriculture*, 90(11):1827-36.
- Jordan CA, Neumann E, Sowers AE. 2013. Electroporation and electrofusion in cell biology. Springer Science & Business Media.
- Katzung BG. 2001. Introduction to autonomic pharmacology. Basic and clinical pharmacology. 13:87-109.

- Khodadadi M, MOTAMED RO, Jahani M, Dorri H. 2012. The survey on Bacterial and fungi contamination of herbaceous distillates that distributed in Birjand city.
- Kovačević DB, Putnik P, Dragović-Uzelac V, Pedisić S, Jambrak AR, Herceg Z. 2016. Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food chemistry*, 190:317-23.
- Lacombe A, Niemira BA, Gurtler JB, Fan X, Sites J, Boyd G et al. 2015. Atmospheric cold plasma inactivation of aerobic microorganisms on blueberries and effects on quality attributes. *Food microbiology*, 46:479-84.
- Mandegary A, Sayyah M, Heidari MR. 2004. Antinociceptive and anti-inflammatory activity of the seed and root extracts of *Ferula gummosa* Boiss in mice and rats. *DARU Journal of Pharmaceutical Sciences*, 12(2):58-62.
- Mills S, Bone K. 2000. Principles and practice of phytotherapy. Modern herbal medicine. Churchill Livingstone.
- Niemira BA, Sites J. 2008. Cold plasma inactivates *Salmonella* Stanley and *Escherichia coli* O157: H7 inoculated on golden delicious apples. *Journal of Food Protection*, 71(7):1357-65.
- Patil S, Moiseev T, Misra N, Cullen P, Mosnier J, Keener K et al. 2014. Influence of high voltage atmospheric cold plasma process parameters and role of relative humidity on inactivation of *Bacillus atrophaeus* spores inside a sealed package. *Journal of Hospital Infection*, 88(3):162-9.
- Paixão L, Fonteles TV, Oliveira VS, Fernandes FA, Rodrigues S. 2019. Cold plasma effects on functional compounds of siriguela juice. *Food and Bioprocess Technology*, 12(1):110-21.
- Pankaj SK, Wan Z, Keener KM. 2018. Effects of cold plasma on food quality: A review. *Foods*, 7(1):4.
- Philip N, Saoudi B, Crevier M-C, Moisan M, Barbeau J, Pelletier J. 2002. The respective roles of UV photons and oxygen atoms in plasma sterilization at reduced gas pressure: the case of N/sub 2/-O/sub 2/-mixtures. *IEEE Transactions on Plasma Science*, 30(4):1429-36.
- Sádecká J. 2007. Irradiation of spices—a review. *Czech J Food Sci*, 25(5):231-42.
- Salarieh S, Dorrnanian D. 2013. Sterilization of turmeric by atmospheric pressure dielectric barrier discharge plasma. *Plasma Science and Technology*, 15(11):1122.
- Sospedra I, Soriano JM, Mañes J. 2010. Assessment of the microbiological safety of dried spices and herbs commercialized in Spain. *Plant foods for human nutrition*, 65(4):364-8.