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Effects of cold plasma treatment on antioxidants activity, phenolic contents and shelf life of fresh and dried walnut (Juglans regia L.) cultivars during storage

Maryam Amini*, Mahmood Ghoranneviss

Plasma Physics Research Center, Science and Research Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT

The present study investigated the effect of cold plasma on the stability of the total phenolic content, antioxidant activity, and inhibition of microbial growth in dried and fresh walnut cultivars during storage. In this study four walnut cultivars named Mazandaran, Toyeserkan, Taleghan and Shahmirzad was used for comparison. The results indicated that, although 11 min of plasma jet treatment caused complete elimination of *Aspergillus flavus* that had been inoculated onto fresh walnut cultivars, the amount of decrease on the inoculated walnuts was different for different cultivars. This difference may be caused by the differing volumes and thickness of the walnut cultivars. In addition, 10 min of plasma jet treatment eliminated *Aspergillus flavus* from the dried walnuts. After 15 and 30 d of storage (4 °C), the number of survivors in the samples for 11 min was negligible. The 11 min plasma jet treatment had no effect on the total phenolic content and antioxidant activity of the dried and fresh walnuts. The effect of plasma jet treatment on the total phenolic and antioxidant activity of all cultivars was the same. An increase in the total phenolic content and antioxidant activity after 15 and 30 d of storage (4 °C) was observed in the control and treated samples. Changes in the total phenolic content and antioxidant activity in the control sample and treated walnuts showed a similar pattern.

1. Introduction

Today, the worldwide walnut production is increased, because of increasing in consumers demand for this nut. World production of walnuts is about 1,500,000 metric Tons and China, the United States and Iran are the major producers of walnut. Among spices of walnuts, the Persian walnut (J. regia L.) is the most cultivated species and the most commercially important. Unfortunately, contamination of walnuts by aflatoxins produced from the fungus *Aspergillus spp*. is a serious problem because of the potential threat to health. Inhibition of fungus before the toxins are produced is more important than the removal of toxins once produced. It is of interest to develop methods or processes to reduce or completely eliminate fungus before aflatoxins are produced during storage. Cold Plasma is ionized gases that contain ions, electrons, reactive neutral species (radicals, and excited atoms and molecules). Cold plasma has been studied for various fresh products such as fruits and vegetables (Kim, Lee, & Min, 2014; Ziuzina, Patil, Cullen, Keener, & Bourke.P, 2014.) Basaran, Basaran-Akgul, and Oksuz (2008) studied elimination *Aspergillus parasiticus* from hazelnuts, peanuts, and pistachio nuts surface with low pressure cold plasma treatment. There are no studies about decontamination of walnuts by cold plasma. Moreover the effect of cold plasma on different cultivars had not been considered to date. Al-Bachir and Sass (1987) reported that after 1.0 kGy gamma irradiation of 12 grape varieties different results were achieved and this dose was not optimum to all varieties. They reported that the natural characteristics such as thickness of skin and color of the grapes cause this difference. It is important to consider cultivars for using cold plasma technology in industrial scale because it is possible that after using a cold plasma device with different parameters (input power, voltage and gas and treatment time) the decontamination is not done completely and because of different genotype or characterize of different cultivars, different changes in nutritional value of an agricultural product was observed. Harrison, and Were. (2007) reported that gamma irradiation with dose 4 and above cause increased total phenolics in almond skins purchased from Blue Diamond Growers and, likewise, doses of 12.7 and above irradiated on almond skins purchased from

* Corresponding author.
E-mail address: Maryam.amini63@yahoo.com (M. Amini).

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Campos Brothers. For using cold plasma technology in industrial scale checking the plasma-quality of object is important to validate cold plasma as a new preservation method. Kernels are an excellent source of phenolic and possess high antioxidant capacity (Halvorsen et al., 2006; Kornsteiner, Wagner, & Elmadfa, 2006). Then it is important to evaluate the effect of cold plasma on total phenolic content and total antioxidant capacity of walnuts immediately after treatment and after storage. There is only a study on the effect of cold plasma on antioxidants activity of kiwi fruit (Ramazzina et al., 2015). Moreover, there is a lack of data concerning the effect of cold plasma after storage. During storage, *antioxidants* and *phenolics* are prone to oxidation resulting to nutritional or sensory deterioration (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2000). The aims of this study were to (i) Determine efficiency of plasma jet to inactivate *A. flavous* inoculated in fresh and dried walnut cultivars (ii) Determine the effect of cold plasma on total phenolic content and antioxidant activity content of fresh and dried walnut cultivars. (iii) Evaluating the stability of the Total Phenolic content and antioxidant activity of the walnuts after 15 and 30 days of storage.

2. Material and methods

2.1. Sample preparation

Walnuts fruits (J. regia L.) were collected from commercial plantations at the same time in Toyserkan, Taleghan, Mazandaran and Shahmirzad of Iran. Toyserkan cities is located at the foot of the central Zagros mountain. The mean annual rainfall ranges between 320 and 350 mm and this region has semi-arid climate. Mazandaran is located in the south of Iran. It has hyrcanian climate and the mean value of annual precipitation is about 1600 mm. Shahmirzad is located on the southern slopes of the Alborz Mountains. Shahmirzad's walnut orchard with the size of 700 ha is noted by the UN, Food and Agriculture Organization, as the largest of its kind in the world. The mean annual rainfall ranges of Shahmirazed is 129 mm and this region has warm climate. Taleghan is located along Alborz mountain Range and it has mountainous climate The mean annual rainfall ranges 450–500 mm and this region has warm climate. Immediately after harvest, fruit were hulled with tap water and unshelled walnuts were used for experiments. Half of the fresh walnuts were selected randomly as a fresh sample while the other half were dried at 36 °C for 24 h and examined as a dried walnut. Fresh and dried unshelled kernels is divided in the middle and put on petri dishes. The dried and fresh samples were divided into 2 groups. One group was control and the other was treated by the plasma jet at 3,5,7,9,10 and 11 min. Treated and untreated samples divided into 3 groups. One group is analyzed for determination of microbial count, total phenolic content and antioxidant activity immediately. The remaining two groups were placed in open plastic pots at 4 °C with 90% R.H and Sorted for 15 days and 30 days then analyzed separately.

2.2. Microbial contamination

Aspergillus flavus Persian Type Culture Collection (PTCC-5004) was obtained from the culture collection at Iran Institute of Industrial and Scientific Research. The *A. flavous was* cultivated on potato dextrose agar (PDA; Merck, Mexico) slants for 10 days at 25 °C until sporulation occurred. The spores were harvested by adding 10 mL of 1 mL/1000 mL Tween 80 solution and scraping the spores with a sterile inoculating loop. Number of spores in the suspension was determined using a hemocytometer(AGB Ltd, Dublin, Irland) and the spore density was adjusted by dilution with 0.1% peptone water. The samples were placed in petri dish. The fresh and dried walnuts were surface disinfested with 95% ethanol (Merck, Germany) and drying under a laminar flow hood for several hours to eliminate possible growth of other microorganisms. A 1.0-g suspension of *A. flavous* was inoculated over the whole surface of dried and fresh walnuts using a sterile glass sprayer and then dried in a laminar flow biohazard hood. 25 fresh walnuts and 25 dried walnuts were treated by the cold plasma systems at different treatment time and 10 fresh walnut and 10 dried walnuts were kept untreated as a control.

2.2.1. Determination of length, width, thickness and volume

To determine the volume of kernels, 100 kernels were randomly selected and their length (L), width (W) and thickness (T) were measured using a digital versnier caliper (Mitutoyo, Japan). (With an accuracy of 0.01 mm). The volume was calculated by following formula

$$V = (\Pi/6)\, LWT$$

2.3. Determination of total phenolic concentration (TP) and antioxidant activity (AA)

The total phenolic content was determined according to Folin-Ciocalteu colourimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999). After addition of the Folin–Ciocalteu reagent (Merck,KGa Germany) the absorbance at 760 nm was measured using a spectrophotometer (Pharmacia LKB, Uppsala, Sweden). Gallic acid was used as a standard and the results are expressed in mg Gallic Acid Equivalents (GAE) g/dry weight. Antioxidant activity was determined according to both ferric reducing antioxidant power or FRAP (Benzie & Strain, 1996) and radical scavenging capacity (DPPH) (Brand-Williams, Cuvelier, & Berset, 1995) assays. For the FRAP assay, 0.1 mL diluted extract was added in 3 mL of FRAP reagent [300 mM acetate buffer, pH 3.6; 10 mM TPTZ (2, 4, 6-tripyridyls- triazine)(Merck, Darmstadt-Germany) in 40 m M HCl(Merck, Germany); 20 m M FeCl3 ·6H2O(Merck KGaA, Germany); in 10:1:1 (v/v)] preheated to 37 °C. The mixture was incubated at 37 °C for 30 min then the absorbance was measured at 593 nm. For the DPPH assay, 0.1 mL diluted sample of the extract was added to 4 mL DPPH solution (2, 2-diphenyl-1-picryhydrazyl (Merck, Germany), 60 M in MeOH (Merck, Germany). The absorbance was measured at 515 nm.

2.4. Experimental set up

The plasma jet consisted of a Pyrex tube (ID: 3 mm and OD: 5 mm) and power electrode (copper wire) which was wrapped around the glass tube as nozzle. The power of the electrode was driven by a 12-KHz pulsed dc 15 KV high voltage power supply. For evaluating the effect of the plasma jet on food, the feeding gases were 99.999% pure argon (Ar) with 1 L/min gas flow rate and distance between the samples and the nozzle tip was 1.5 cm that was kept unchanged.

2.5. Statistical analysis

SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. Average values from triplicate experiments were obtained and One-way analysis of variance (ANOVA) followed by Duncan's test was performed to determine the differences in mean values of achieved data from different treatment times. Statistical differences were considered as significant at $P < 0.05$.