In package control of *Rhyzopertha dominica* in wheat using a continuous atmospheric jet cold plasma system

Reshma Vadakhe Madathil*, Ranjitha Gracy Thirugnanasambandan Kalaivendan †, Anjaly Paul ‡, Mahendran Radhakrishnan §

† St. George’s College, Aruvithra, Kerala, India
‡ Institute of Chemical Technology, Mumbai
§ Indian Institute of Food Processing Technology, Thanjavur, India

* Corresponding Author: mahendran@iifpt.edu.in

Received: 22-02-2021, Revised: 11-05-2021, Accepted: 12-05-2021, Published: 21-05-2021

**Abstract:** Cold plasma is recognized and explored for a plethora of applications in the food and agricultural industry. This study investigated the influence of a continuous atmospheric pressure non-thermal jet plasma system on the mortality of *Rhyzopertha dominica* adults in whole wheat kernels and the changes in the milling and physicochemical attributes of the treated whole wheat. Air-filled packets of whole wheat kernels were artificially infested with *R. dominica* adults. The packages were carried by a continuous conveyor belt and treated with plasma at voltages ranging from 44-47 kV for 4-7 min. The mortality was determined after 24 h and milling yield, particle size, proximate composition, and color of plasma-treated and untreated wheat grains were also evaluated. The maximum mortality was 88.33% at 47 kV for 7 min. The milling yield, protein, and fiber content of wheat were enhanced with plasma treatment significantly. Thus the continuous atmospheric pressure jet plasma used in this study could be one of the practically implementable emerging techniques for the commercial disinfestation of packaged food products.

**Keywords:** Atmospheric pressure jet plasma, Disinfestation, Milling properties, *Rhyzopertha dominica*, Wheat.

**1. Introduction**

Wheat is one of the major cereal crops after rice and maize, with over 767 million tons being harvested annually and serves as an essential part of the diet for most of the people in the world. However, one of the major problems causing considerable losses during the production and storage of these grains is the infestation by insect pests. The stored product pests not only
initiates the qualitative losses through their cast skins, excreta, fragments, and other by-products but also cause quantitative losses by feeding. Infestation also decreases nutritional value, reduces seed germination, and causes alteration of chemical composition (increase in moisture, free fatty acid levels, and non-protein nitrogen content due to metabolic action of insects; and decrease in protein content) [1,2]. The lesser grain borer *Rhyzopertha dominica* is a primary pest causing significant economic damage to the wheat grains. *R. dominica* is one of the most destructive pests and feeds on the endosperm of the seeds. The larvae and adults of *R. dominica* can penetrate inside the kernel, causing internal injury to wheat, thereby resulting in substantial weight loss and quality degradation [3]. A study conducted on the quality of the flour from *R. dominica* infested wheat concluded that infestation causes adverse changes in the flour properties such as water absorption, dough development time, mixing, and dough stability [4]. Thus the efficient control of *R. dominica* affecting the grain has become a serious concern throughout the world.

In recent years the technology has marked advancements in the study of disinfestation of stored grain pests, finding alternative ways to control them [5]. The traditional approaches adopted for the control of *R. dominica* in wheat rely on the use of synthetic pesticides and fumigants like phosphine and methyl bromide [6] which have a negative impact such as producing toxic residues, pollution to the environment, and resistance development by the insects. To displace the use of pesticides and fumigants against *R. dominica*, the use of organic disinfectants such as diatomaceous earth [7] and plant products have been explored widely [8]. However, they exhibit delayed response towards the control of stored product pests, making the commercial applications cumbersome.

Novel nonthermal approaches like ozonation [9], gamma irradiation [10] and cold plasma are emerging disinfestation techniques. Among which cold plasma is a potential disinfestation method against a wider range of insect species like *Myzus persicae* [11], *Frankliniella occidentalis* (Pergande), *Frankliniella fusca* (Hinds), *Aedes albopictus* (Skuse), *Tetranychus urticae*, *Blattella germanica* (L.) [12], *Plodia interpunctella* [13] and *Tribolium castaneum* [14,15].

Plasma composed of free radicals, reactive oxygen and nitrogen species, UV radiation, atoms in their ground, and excited states engenders destructive effects to the insects, thereby resulting in effective insect control. Despite the above works stating the potentiality of cold plasma for disinfestation, the effect of cold plasma on the mortality of insects in packed grains and on the grain quality is yet to be studied. This helps in addressing the lack of feasibility in cold plasma application for the disinfestation of packed whole grains. Thus this study aimed to investigate the effect of atmospheric pressure non-thermal jet plasma on mortality of *Rhyzopertha dominica* in whole wheat and its effect on wheat quality attributes such as milling yield, flour particle size, color, and proximate composition.
2. Materials and Methods

2.1 Culturing Rhyzopertha dominica adults:

Adults of *R. dominica* were collected from the already infested stored wheat stocks. The segregated insect adults were reared on whole wheat of samba variety (*Triticum dicoccum*) procured from the local market, Thanjavur, India. The wheat grains (7.83±0.24% moisture content) used for culturing and plasma treatment were assured to be disinfested earlier by storing it at -17 °C for two days, followed with air drying to prevent the risk of mold growth [16]. The adults were nurtured on the uninfested whole wheat kernels in a plastic container covered with muslin cloth providing aeration and incubated at 30±2 °C at 70±5% RH. After 20 days of rearing [17], the added adults were isolated from the stock culture, leaving the eggs, larvae, and instars in the same stock to yield newly emerged adults needed for the experiment.

2.2 Raw material preparation:

Forty grams of the sound wheat grains were prepensely infested with 20 healthy newly emerged *R. dominica* adults [18] and air packed in a high oxygen barrier, oriented polypropylene films. The packs were deliberately filled with air (constant headspace volume of 32 cm$^3$) to enhance the impact of plasma on insect mortality as the formation of reactive species depends on the volume of air. The packs were sealed in such a way to facilitate their movement through the conveyors between the electrodes without making contact.

2.3 Atmospheric pressure DBD plasma treatment:

The plasma reactor used for the experiment (figure.1) was developed at the Indian Institute of Food Processing Technology, Tamilnadu, India, which operates at atmospheric pressure using atmospheric air as the source of the discharge. The sample moved in a conveyor between the aluminum electrodes upon which a 2 mm thin layer of glass dielectric was provided for the Dielectric Barrier Discharge (DBD). The distance between the electrodes was kept constant at 5 cm. The electrodes were charged with high AC voltage ranging from 0-100 kV with 0-50 mA. The provision of a conveyor belt moving across the electrodes make the system feasible for continuous plasma treatment. In the current study, the air packed wheat sample was positioned between the aluminum electrodes and treated with plasma at four different voltages ranging from 44-47 kV for four exposure times of 4-7 min and studied for mortality and physicochemical attributes. All the treatments were done in triplicates.

2.4 Mortality determination:

The plasma-treated wheat samples were checked for mortality along with the untreated wheat sample kept as control after 24 h of acclimatization. The packs were cut open and inspected for dead and live insects. The mortality of the insects was calculated using eq.1 [19]. The insects showing no response to stimuli were considered to be dead. The treated wheat samples were further analyzed for other physicochemical attributes.
Mortality (%) = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100 \quad \text{(eq.1)}

Figure 1. Schematic diagram of the continuous atmospheric pressure plasma reactor

2.5 Analysis of physicochemical attributes of wheat:

2.5.1 Nutritional profile

The proximate composition of the plasma treated and untreated wheat grains were analyzed. Moisture content was determined gravimetrically by measuring the weight of the sample before and after keeping it in the hot air oven at 130°C for 1 h [20]. The Kjeldahl method was employed to determine the total nitrogen and crude protein. Total fat content was estimated after extraction with hexane using a Soxhlet apparatus. The gravimetric incineration technique was adapted to determine the total ash content. The crude fiber was estimated from the weight of the defatted sample after acid and alkali digestion [21]. Carbohydrate content was calculated through mass balancing the other proximate components.

2.5.2 Milling yield and particle size

The plasma-treated and untreated wheat samples were analyzed for milling yield after subjecting to hammer mill with a constant feed rate of 240 g/min. The milled flour was then categorized into fractions like shorts, bran, and fines of different particle sizes using a vibratory sieve tester (INSIF, India). The flour of particles passing through the 135 μm sieve was
considered the desired fine flour contributing to the milling yield [22]. The milled samples were used to find the average particle size using the fineness modulus (FM) equation (eq.2) [23].

\[
\text{Average particle size} = 0.135 \times 1.366^{\text{FM}}
\]  
(eq.2)

2.5.3 Color:

Hunter lab ColorFlex EZ, 45/00 Color Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, Virginia, USA) was used to measure the color of wheat flour obtained from plasma-treated whole wheat grains. Since the color of flour is an essential indication of wheat flour quality, the effect of cold plasma on the whole wheat kernel color was disregarded. Initially, the colorimeter was calibrated with the black and white tiles. The lightness (L*), redness (a*), and yellowness (b*) of the flour were recorded, and the color difference (\(\Delta E\)) between the untreated and plasma-treated wheat flour was calculated as given in Eq. 3 [24].

\[
\Delta E = \sqrt{\left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2 + \left(\Delta L^*\right)^2}
\]  
(eq.3)

Where,

\(\Delta a^*\) – Change in redness of treated flour

\(\Delta b^*\) – Change in yellowness of treated flour

\(\Delta L^*\) – Change in the lightness of treated flour

2.6 Statistical analysis

All the experiments were done in triplicates. Statistical analysis was done with SPSS statistical software (SPSS Inc, Chicago, USA, Version 20.0). Two way ANOVA was performed to find the effect of voltage and time on the mortality of insects, and one-way ANOVA was done separately at different times to study the variations among voltages. Significant differences in all the analyzed physicochemical properties and the percentage of mortality with the application of plasma treatment were analyzed using Tukey’s test at a 95% confidence limit (p < 0.05).

3. Results and Discussion

3.1 Effect of plasma on the mortality of R. dominica adults

The effect of process parameters such as plasma voltage and exposure time on the percentage mortality of \(R. dominica\) adults were investigated and the obtained results were given in figure 2. It was observed that the number of dead insects significantly (p < 0.05) augmented with the increased plasma voltage and exposure time. The mortality was increasing from 3.33% at 44 kV for 4 min treatment to a maximum of 88.33% at the highest process condition of 47 kV for 7 min. There is an amalgamation of mechanisms involved in the insecticidal activity of plasma. The predominant reactive oxygen species, ozone in plasma, decrease \(R. dominica\) adults' respiration rate incessantly without retrieval, which tends to instigate asphyxiation of insects [25]. Further, the other oxidative species such as hydrogen peroxide, superoxide anion, hydroxyl
radical, hydroperoxyl radicals interact with the constituents of the insect to yield oxidized radical intermediates which will propagate the radical reactions further to exacerbate the structural and metabolic integrity of the cells [26]. These intermediates may also indulge in the untangling of DNA strands and damage the DNA, ultimately resulting in insect mortality [27]. The charged particles produced during the plasma generation gets accumulated on the surface of the membrane, eliciting an electrostatic force, and thus contriving cell rupture [28]. Intense bombardments of free radicals with insects create surface lesions, leading to the perpetual rupture of the living cells [13]. Altogether, these causative agents of insect disinfection in plasma would be intensified at process conditions such as 47 kV for 7 min, resulting in the highest mortality rate of about 88.33%.

![Figure 2. Effect of plasma process parameters on mortality of Rhyzopertha dominica adults](image)

### 3.2 Effect of plasma on physicochemical attributes of the wheat kernel

The physicochemical attributes of the plasma-treated wheat grains after milling were studied, emphasizing its nutritional and milling properties.

#### 3.2.1 Nutritional profile

The production of free radicals and reactive species during plasma treatment could interrupt the proximate composition through oxidative reactions. Thus, it is essential to investigate the effect of cold plasma treatment on the same. In the present study, the nutritional composition of wheat grains was significantly (p<0.05) affected (table 1) with the plasma treatment excluding the ash content particularly at the highest operating parameters,
i.e., with the plasma voltage of 47 kV for 7 min treatment time. Delineating further, protein the significant component of interest in wheat was increased with the plasma treatment from 13.6% to about 16.8% consistently. This might be due to the formation of free amino acids produced on the disruption of intricate peptide chains by the reactive species [29]. Also, the instigated enzymatic activities may indulge in an increase in the protein content. Similar results were observed by [30] in the case of soybean. Further, there are possibilities that the constituents of the protein secondary structures such as α-helices and β-sheets would get altered by plasma treatment. [31] studied the dough rheology of wheat flour after atmospheric pressure DBD plasma treatment and observed an increase in viscoelastic properties at increasing voltage and time.

Similarly, the fat content, which is a prominent oxidation sensitive component, was significantly (p<0.05) decreased at plasma voltages of 44 and 45 kV. Later, it got slightly increased at the higher plasma voltages of 46 and 47 kV. Though this change was ambiguous, the fat content remained in the range of normal wheat grains ~ 2%. A variation in the pattern will be due to the changes in specific wheat grains. Usually, on plasma treatment, the fat molecules get oxidized to
yield lipid oxidation products like malondialdehyde and alkenals [32], which may reduce the fat content noticeably. Over and above the carbohydrates were reduced significantly (p<0.05) from 75.88% to 69.40% with the plasma treatment, because the storage polysaccharide content, i.e., the starch might get reduced when exposed to oxidative stress [33].

The moisture content of plasma-treated wheat was increased from 7.83% to the maximum of 11.67% significantly (p<0.05) at all plasma voltages but in a non-linear way. The oxidative species of plasma, on interaction with the nutritional elements of grains, produce vapors, which tend to increase the vapor pressure of the grains [34]. The increased vapor pressure creates a vapor pressure gradient, which is a driving force for moisture loss of grain to the ambient [35]. This would reduce the moisture content of the grain. However, in the current study, the airtight sealed package precludes the escape of water vapor to the outside, leading to condensation on the grains, thus increasing the moisture content. Even then, the moisture content of the plasma-treated grains was in the range of ~ 12%, which is below the safe storage moisture content [36]. However, then again, this rise in moisture content was correspondent to the original moisture content of wheat grains, which necessitates the investigation of the effect of cold plasma on wheat quality with varying grain initial moisture content. The plasma causing surface modification weakens the fiber strength of the grain [37], thereby increasing the accessibility. This results in the increased crude fiber content of plasma-treated samples significantly (p<0.05). Since the mineral content of wheat is minimal, there were no significant changes observed in the ash content of plasma-treated samples as compared to the control. Despite the observed changes in the proximate composition of wheat grains with plasma treatment, a detailed study is required to understand and confirm the reactions involved.

3.2.2 Milling yield and average particle size

Milling properties of the wheat grain, such as milling yield and average particle size of the flour, play a vital role in deciding the miller's acceptance of the grains pertained for a specific final product [38]. The milling yield and average particle size of the wheat flour obtained from plasma-treated and control wheat grains were given in Figure 3. It was observed that the plasma treatment did not cause any significant (p<0.05) changes in these parameters. However, milling
yield was increased marginally with the plasma treatment of 47 kV exclusively with the treatment time of 5-7 min from 52.4% to 57.3%.

Figure 3. Milling properties of plasma treated wheat grains a) Milling yield and b) Particle size
This might be attributed to the reactive oxygen species and the energized radicals which might interact with arabinoxylans [39] and other structural polysaccharides of the wheat kernels, undermining the endosperm resistance towards rupture [40]. This facilitates breakage of wheat kernels with minimum impact force since the milling power required depends on the hardness of the wheat kernel [41], resulting in increased milling yield with the increase in the concentration of reactive species at higher plasma voltage and treatment time. Besides, the average particle size of the milled flour was around 0.34 mm, which is moderately higher than the optimum particle size of whole wheat flour, i.e., < 0.20 mm [42]. This might be because of milling a lesser amount of wheat underrun the capacity of the mill. Nevertheless, these results indicate the applicability of the technique to obtain a superior yield of wheat flour of about > 60% [43] with optimum particle size.

3.2.3 Color

The physical property color is the predominant product parameter deciding the consumer acceptance of the commodity.

Table 2. Color of plasma treated and untreated wheat grains

| Plasma voltage (kV) | Treatment time (min) | Lightness value (L*) | Redness value (a*) | Yellowness value (b*) | Del E value (ΔE) |
|---------------------|----------------------|----------------------|-------------------|----------------------|-----------------|
| Control             |                      | 78.86 ± 0.10 \( ^a^A \) | 3.05 ± 0.04 \( ^a^A \) | 15.27 ± 0.09 \( ^a^A \) | -               |
| 4                   | 4                    | 79.40 ± 0.23 \( ^b^B \) | 3.02 ± 0.03 \( ^a^A \) | 15.35 ± 0.05 \( ^a^A \) | 0.58 ± 0.27 \( ^a^A \) |
|                     | 5                    | 79.18 ± 0.20 \( ^b^B \) | 3.25 ± 0.05 \( ^a^A \) | 15.57 ± 0.11 \( ^a^A \) | 0.38 ± 0.03 \( ^a^A \) |
|                     | 6                    | 79.43 ± 0.21 \( ^b^B \) | 2.93 ± 0.05 \( ^a^A \) | 15.00 ± 0.26 \( ^a^A \) | 0.85 ± 0.19 \( ^a^A \) |
|                     | 7                    | 79.23 ± 0.24 \( ^b^B \) | 3.26 ± 0.02 \( ^a^A \) | 15.91 ± 0.06 \( ^a^A \) | 0.70 ± 0.10 \( ^a^A \) |
| 45                  | 4                    | 79.14 ± 0.30 \( ^b^B \) | 3.09 ± 0.07 \( ^a^A \) | 15.64 ± 0.19 \( ^a^A \) | 0.53 ± 0.12 \( ^a^A \) |
|                     | 5                    | 79.50 ± 0.42 \( ^b^B \) | 3.05 ± 0.12 \( ^a^A \) | 15.46 ± 0.28 \( ^a^A \) | 0.83 ± 0.26 \( ^a^A \) |
|                     | 6                    | 79.55 ± 0.24 \( ^b^B \) | 3.09 ± 0.02 \( ^a^A \) | 15.36 ± 0.09 \( ^a^A \) | 0.43 ± 0.10 \( ^a^A \) |
|                     | 7                    | 79.53 ± 0.42 \( ^b^B \) | 3.04 ± 0.05 \( ^a^A \) | 15.42 ± 0.18 \( ^a^A \) | 0.57 ± 0.10 \( ^a^A \) |
| 46                  | 4                    | 78.84 ± 0.18 \( ^b^B \) | 3.42 ± 0.35 \( ^a^A \) | 15.73 ± 0.11 \( ^a^A \) | 0.89 ± 0.06 \( ^a^A \) |
|                     | 5                    | 79.64 ± 0.28 \( ^b^B \) | 3.03 ± 0.01 \( ^a^A \) | 15.34 ± 0.05 \( ^a^A \) | 0.71 ± 0.12 \( ^a^A \) |
|                     | 6                    | 79.61 ± 0.15 \( ^b^B \) | 3.21 ± 0.03 \( ^a^A \) | 16.00 ± 0.03 \( ^a^A \) | 0.89 ± 0.09 \( ^a^A \) |
|                     | 7                    | 79.84 ± 0.20 \( ^b^B \) | 2.97 ± 0.03 \( ^a^A \) | 15.12 ± 0.07 \( ^a^A \) | 1.09 ± 0.21 \( ^a^A \) |
4. Conclusion

The effect of continuous atmospheric pressure jet plasma on the mortality of *Rhyzopertha dominica* adults in whole wheat kernels was investigated along with physicochemical and milling properties of treated wheat grains. The highest percentage of mortality of 88.33% was obtained at the plasma voltage of 47 kV for 7 min of treatment. A 100% of mortality could be attained with a slight increase in the process conditions for the whole wheat kernels, which will be examined in future studies. The study on milling and physicochemical properties encountered minimal changes after plasma treatment, which are neither detrimental for the consumer nor deficit for the processor. Thus continuous atmospheric pressure nonthermal plasma can be adopted as an effective and environmentally friendly disinfestation technique in an integrated pest management (IPM) program. Further studies can be done on other primary physicochemical attributes, such as alpha-amylase activity and dough rheology. Also, efforts must be taken to enunciate the mechanism of plasma disinfestation on different insect species causing distinct storage losses.

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Acknowledgements: Authors acknowledge the funding support of ICAR-EMF, Grant/Award Number: (A. Enng.2(10)/2015-AE); and IIFPT, MoFPI.

Conflict of interest: NIL.

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