Journal of Food Quality

Nonthermal Plasma for Food Quality and Safety

Lead Guest Editor: Vladimír Scholtz
Guest Editors: Josef Khun and Božena Šerá

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Editorial Board

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Editorial

Nonthermal Plasma for Food Quality and Safety

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Although the microbicidal effects of nonthermal plasma (NTP-the cold ionised gas) were reported more than fifteen years ago, we are still waiting for its wider application in practice. Though there are lots of published scientific papers describing the reduction and inactivation of pathogenic microorganisms (e.g., bacteria and spores) by NTP exercisable in microbiology, in medicine for disinfection of thermolabil instruments and therapy of infections, and in food processing for microbial decontamination of food or production components, their application in practice is still not generally accepted. Nevertheless, it seems the final step of apparatus upscaling and application in practice should become directly.

NTP technology is useable above all in the food industry. NTP can inactivate decontaminated surfaces of various food products and semifinished food products, yet the effect is relatively gentle on surfaces being decontaminated. NTP provides sufficient efficiency accompanied by minimal damage and minimal impact on the quality of processed foods or thermosensitive packaging materials. Thus far, it appears that, at least in some cases, there is minimal damage of antioxidants in food, and the content of substance residues is minimized after the plasma treatment. It is a usable effect that NTP may start better germination and early growth in many agricultural crop seeds.

By our experience from the food and agriculture industry, one of the main reasons may be the skepticism of producers about the effectivity and suitability of NTP and also their unfamiliarity. Nowadays, when the knowledge of NTP effects is general in the scientific area, our next big deal is to present and propagate the potential of NTP to the public community. This special issue proposes to collect a set of several interesting works to catch the attention of potential applicants. This issue includes five original papers and one review.

The first three papers confirm the potential of NTP to decrease the number of food microflora together with the analysis of the quality of treated food. The various food samples they used are both perishable ones such as chicken meat and onion and durable ones such as rice.

The next article together also with the previous ones pays attention to the food quality affection by NTP. The paper presents the study about their influence on both physical and chemical properties such as color, content of antioxidants, fatty acids, protein, sugar, thermodynamic properties, solubility, and pH.

The last but one article addresses the not yet well-known fact of food functionality affection by NTP presented on cereal flour and dough properties.

As a final article, we offer our review summarizing the results of several studies of NTP treatment of wheat grains as one of the world's most important nutritive. We focus mainly on the possible effects of NTP on wheat rather than on the detailed description of plasma generation and other treatments. Details and description of the plasma effect begin at the surface of wheat grains and continue stepwise through the growth characteristics, metabolisms, and matured plants to the flour as the final food product.

We hope that this special issue contributes to familiarize the awareness of NTP features suitable for food industry and that the gentle reader finds it interesting. We wish you a pleasant reading.

Conflicts of Interest

The editors declare that they have no conflicts of interest.

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Review Article

Effects of Nonthermal Plasma on Wheat Grains and Products

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This paper presents the review of effects of nonthermal plasma (NTP) treatment on both the wheat grains and flour with potential to be applied in practice. The NTP can be used in wheat grain surface disinfection, grain germination and vitality improving, and wheat flour modification and disinfection. NTP causes effective decontamination from bacteria and fungi together with insect pests and causes minimal damage to wheat grains; it inactivates enzymes and enhances the grain shelf life; it enhances the germination and initial state of growth resulting in the increase of final yield. Moreover, the production of qualitatively better dough is also mentioned.

1. Introduction

Nonthermal plasma (NTP) has been constituted in biotechnology as an alternative method for food processing and as an emerging antimicrobial technology for inactivation of undesirable microflora. Moreover, it has been found that NTP affects also other parameters of both treated inorganic and biological objects. In the last decade, NTP treatment of grains has been established as a possible new field of interest. Examples of these approaches may be found in [1–4].

The cereals are the main ingredient of human food, where only grains are used for human nutrition [5, 6]. The grains are consumed either whole or ground to flour, which exhibits different final granulation and/or chemical composition. Wheat is one of the world's leading food crops and is one of the most grown cereals [5] as a source of white wheat products: white bread, toasts, baguettes, hamburgers, croissants, and pizza's dough. The possibilities of NTP treatment of wheat grains in agrotechnical practices have been studied by many scientific teams in the one recent decade. The significance and impact of such studies is undoubtedly significant for better preservation of cereals for nutrition. However, the existing NTP applications for this purpose suffer from inconsistent methodologies, making it impossible to compare and select the optimal methodology. Therefore, our goal here is not to determine the best method, but rather to enumerate the options used so far.

In this review, we would like to present summarized results of several studies of NTP treatment of wheat grains to demonstrate the new possible ways of affecting its properties. We have chosen the wheat grain as one of the world's most important nutritive with wide amount of published studies. Further, it is possible to predicate similar NTP affections of other species of cereals also. In general, for clarity, we want to give up describing the optimization of each NTP source and its many regimes for specific application. Hence, we decided to focus mainly on the possible effects of NTP on wheat rather than on the detailed description of plasma generation and other treatment details. We assume that friendly reader would be interested especially to the list of interesting and curious effects of NTP and that they may find the details in referred works. The description of plasma

effects presented here begins at the surface of wheat grain and stepwise continues through the growth characteristics, metabolisms, and matured plants to the flour as the final food product.

2. Nonthermal Plasma Technology

The term "NTP" typically denotes the state of ionized gas at ambient temperature with dominant collective behavior of charged particles. It has been the subject of many previous reviews and books, e.g., [7–9]. A nice introduction into plasma and its generation is also presented in the book devoted to plasma medicine [10] or in the review [11]. The common ways to get NTP are electrical discharges, which have been also reviewed for many times, e.g., [10–12]. The brief description of the most frequently used approaches is as follows.

Corona discharge (CD) is typically generated by high voltage on sharp electrodes, such as tips, pinpoints, or thin wires. The electric field is formed close to such points, and the active region of corona and plasma generation arises. Corona discharge active region appears only close to the point electrode, and it is limited up to units of mm. Several modifications of corona-based discharges were tested, e.g., by Khun et al. [13].

Dielectric barrier discharge (DBD) is an alternating current discharge burning typically between two electrodes separated by dielectric material, which avoids the charged particle transport between electrodes. The discharge burns due to the alternating polarization of dielectric and by the electric induction only. In contrast to corona, the DBD electrodes may be constructed as planes, and therefore, the plasma area is limited just by the power of high voltage supply. A brief description of this method may be found, e.g., in [14].

Radio frequency (RF) and microwave discharges (MDs) are generated by high frequencies (MHz and GHz) electromagnetic induction or waves in a resonance box. MDs are often used in the basic research of NTP interactions with biomaterials. For further information, see [15].

Plasma jet (PJ) represents a special configuration of previously described discharges. The active particles from the active region are transmitted through the electrode area by flowing auxiliary gas, forming a stream of active particles burning as a small jet. Typical sources are called plasma jet, plasma pen, plasma torch, or plasma needle. They allow the local application and higher powers. For review, see [16].

Cometary DC discharge [17, 18] resembles plasma jet, but needs no auxiliary gas supply. The insertion of insulated metallic grid improves the inactivation efficiency and size of the treated area [19].

Despite the fact that each discharge is unique and therefore the generalization of results is difficult or impossible, there is a prevailing consensus about the possible mechanisms of NTP affection of the treated object. For a brief overview of this topic, see [20]. In the active discharge area, many active particles are generated from the molecules or atoms of discharge atmosphere. In air or similar atmospheres, the nitrogen active particles, often denominated as reactive nitrogen species (RNS), and the oxygen active particles, often denominated as reactive oxygen species (ROS), are generated. The dominant active particles are radicals such as electronically and vibrationally excited oxygen ($O_2{}^*$) and nitrogen ($N_2{}^*$). The active forms such as atomic oxygen (O), singlet oxygen ($^1O_2$), superoxide anion ($O_2{}^-$), atomic nitrogen (N), excited nitrogen ($N_2(A)$) and H_2O^+, OH^- anion, and $OH^\cdot$ are also important. The stable molecules such as ozone O_3, nitric oxide (NO_x), and hydrogen peroxide (H_2O_2) are generated, too. This extensive field has been reviewed several times, e.g., in [21, 22].

3. Wheat Grains after NTP Treatment

The application of various chemical insecticides and fumigants during grain storage has caused numerous problems, including the accumulation of pesticides and fumigant residues in treated grains [23]. Another serious problem is the development of insecticide resistance in stored grain insect pests [24]. Therefore, there has been growing interest in biotechnology research concerning the possible using of some alternative treatments, such as plant extracts [25], gamma irradiation [26] or plant extract and gamma irradiation together [27], laser [28], or NTP treatment [29–31]. Some earlier attempts to describe the effects of NTP on various seeds, including wheat, have been evaluated in the review [32]. In addition to direct plasma exposure, the action of water previously exposed to NTP (so-called plasma-activated water, PAW) was also reported by Kučerová et al. [33]. The PAW improved germination, early development of the seedlings, the content of photosynthetic pigments in the leaves, and soluble protein content in the roots and suppressed the activity of antioxidant enzymes.

The NTP experiments reported in this section have a common general scheme consisting of dry wheat grains exposure for appropriate time, followed by the analysis of resulting properties. For better clarity, the quoted works and their brief content are also summarized in Table 1.

This summary shows that it is very difficult not only to compare individual results but also to determine the optimal methodology for influencing wheat seeds and products. Various authors employed almost all possible plasma sources (except plasma jet), differing in nature and properties. Different experimental conditions were also used, namely, the exposure times ranging from 10 seconds to 45 minutes.

3.1. Surface Decontamination. The surface of the cereal grains can be contaminated by both the microorganisms and germs of various insect pests [34]. The following works indicate NTP to be a promising tool for effective decontamination offering a wide range of possible applications including inactivation of surface microorganisms and germs of insect pests on cereal grains, above all epiphytic bacteria.

The exposure of winter wheat grains to NTP reduces the fungal colonies' number by one order; as reported by Kordas et al. [35], the optimal exposure time was 10 s. Los et al. [36] found this decrease by two orders. Zahoranová et al. [37]

Table 1: Experimental conditions and results of some attempts to influence wheat grains and flour properties.

Discharge description	Exposure time	Results	Paper
DBD, atmospheric pressure, air, 80 kV, 50 Hz	20 min	Bacteria and fungi reduction of 2.5 $\log_{10}$ Grain surface hydrophobicity decreased Minimal or negative influence on germination	[36]
DBD, atmospheric pressure, air, AC 20 kV, 14 kHz	0–120 s	1 $\log_{10}$ reduction of natural bacteria and 2 $\log_{10}$ reduction of filamentous fungi Grain water uptake increased Germination increased, optimum at 30 s, over 70 s lower than control Increase in dry weight and vigor index, optimum for 30 s	[37]
DBD		Significant reduction of microbial contamination No or negative (over 20 min) influence on germination	[38]
DBD, atmospheric pressure, air, AC 10 kV, ca 13 kHz	20 s	Total reduction of larvae of flour beetles *Tribolium confusum* and *Ephestia kuehniella* No significant changes in fat, protein, ash, and moisture content of flour	[41]
DBD, atmospheric pressure, air, 17 kV, 50 Hz	4 min	Improvement of water uptake of grain surface Germination potential, germination index, germination rate, and vigor index increased Shoot length, root length, dry weight, and fresh weight increased Penetrating of active species into grains improves soluble protein content and α-amylase activity	[44]
DBD, atmospheric pressure, air, AC 13 kV, 50 Hz	13 min	Etching effect on the grain surface; improvement of water uptake Germination potential, germination rate, germination index, and vigor index increased Root length, shoot length, fresh weight, and dry weight of the seedlings increased, optimum at 7 min Enhanced the osmotic-adjustment products and proline and soluble sugar contents	[45]
DBD, atmospheric pressure, air, AC 15 kV in amplitude, 50 Hz	5–30 min	Grain wettability increased Minimal influence on germination Longer roots and sprouts and heavier roots increase of the R/S ratio	[47]
DBD, atmospheric pressure, air, AC 18 kV amplitude, 50 Hz	5–45 min	Improved the germination rate, speed of germination, and speed of growing in the early stage; over 30 min properties decreased under untreated Improved length of roots and sprout, vigor index, number of roots; over 30 min decreased under control	[61]
DBD, low pressure 10 torr, Ar/O_2 and Ar/Air, 5–10 kV, 3–8 kHz	90 s	Improved germination rate and seedling vigor Decrease of root length and root dry weight; increase of shoot length and shoot dry weight Increase of soluble proteins in both roots and shoots	[42]
DBD atmospheric pressure, air, 10 kV, 6 kHz and up to 24 kV, 50–Hz	5–35 min	Reduction of lipase and lipoxygenase enzymes activity; enhance the shelf life of grains	[63]
DBD, variable gas or air, 0.5–3 kV, 50 Hz, from low pressure under 1 mbar to atmospheric pressure	3 min	100% mortality of *Tribolium castaneum* beetle No significant changes in color of flour	[66]
DBD atmospheric pressure, air, 60–70 kV	5 and 10 min	Elastic and viscous moduli of dough from strong wheat flour increased; no variation in the dissipation factor tan δ; improvement of the dough strength	[2]
DBD at atmospheric pressure, 10 kV at 50 Hz	3 min	Reduced development of *Rhizopus nigricans* fungal disease	[39]

Table 1: Continued.

Discharge description	Exposure time	Results	Paper
DBD at 0–50 kV and 50 Hz in the Ar, N_2, air, or O_2 atmosphere	1–19 min	Cleaning of the seeds surface Germination potential increased Etching effects on the seed coat Shoot and root length increased Increase of soluble protein production	[59]
DBD in air, 80 kV, 50 Hz	30, 60, or 180 s	Germination rate enhanced Positive effects on seedling growth Changes of seed surface in seed pH, nitrites, nitrates, and malondialdehyde content	[36]
Not specified, probably DBD, atmospheric air	120 s	No change in the total count of aerobic bacteria and mould in flour Total free fatty acids and phospholipids reduced; some oxidation markers increased Treated flour did produce a stronger dough	[67]
DBD at 6–10 kV, 5–15 kHz in the Ar at atmospheric pressure	0–60 min	Inactivation of bacterial *G. stearothermophilus* endospores	[40]
Low-pressure plasma	15 s	Spike length and number of grains per ear improved; grain weight increased by 1%	[65]
Low pressure 140 Pa·MW discharge	3–40 min	Longitudinal cracks and fast wetting of grain surface Germination enhanced Shoot phenolic compounds increased	[43]
Plasma discharge at frequency 3109 MHz, power 60 W, 80 W, and 100 W, helium atmosphere	15 s	Improving seed germination potential and germination rate Improving plant height, root length, and fresh weight Higher chlorophyll content, nitrogen, and moisture content	[58]
CD, atmospheric pressure, air, AC 8 kV, 0.1–83 kHz	10 s	One-order decrease of fungal colonies No significant differences on lengths of the root and shoot and on weight of the seedling No significant differences in dry matter of plants' root length decreased to cca one-half	[35]
Gliding arc discharge, atmospheric pressure, H_2O/air, H_2O/O_2, H_2O/O_2/air, 5 kV	3–15 min	Grain surfaces more rough; enhanced water permeability into the grains Positive (till 6 min) and negative (over 6 min) influence on germination and plant length Increase in total number of grain per spike, grain weight, and yield	[48]
Glow discharge, 1–6 kV, 3–5 kHz, low pressure 1.3 kPa, air, and air + O_2	3–15 min	Enhancement of water absorption property; increased germination rate Increase of dry weight, spike length, number of spikelet, and number of grains Yield increased by ~20% from plants	[46]
RF 13.56 MHz, air or air + He mixture, low pressure 30–200 Pa	90 s	Germination increased Lengths of root and shoot and dry weight increased	[60]
Plasma-activated water produced by a transient spark discharge	Activation time 1–40 min	Improved germination and development of the seedlings Improved content of photosynthetic pigments in the leaves and soluble protein in the roots Suppressed activity of antioxidant enzymes	[33]

focused their experiments not only on fungi but also on microorganisms in general. They found that NTP treatment leads to a significant reduction of epiphytic bacteria and phytopathogenic and toxinogenic filamentous fungi by two orders. A significant reduction of microbial contamination on wheat and barley grains achieved after NTP exposure was reported also in [38]. Rusu et al. [39] exposed wheat seeds contaminated with *Rhizopus nigricans* with DBD atmospheric pressure plasma for 3 minutes and observed an earlier development of the fungal disease for the control seeds group in comparison with the plasma-treated seeds, a cleaning effect on the seeds surface. Butscher et al. [40] used

argon atmospheric plasma for inactivation of *Geobacillus stearothermophilus* endospores for artificially contaminated wheat grains and polypropylene model substrates. While smooth substrates were decontaminated effectively with reduction of more than 4 magnitudes, the reduction on grain surface was of one magnitude only. The authors considered that the endospores are shielded from plasma-generated reactive species by uneven surface, loose pieces of bran, and the ventral furrow of wheat grains.

Insects are important pests; their decontamination has been reported by Shahrzad et al. [41], who treated 2-3 instar larvae among wheat grains. The mean percentage of mortality reached 100% after 20 s for both the pests called confused flour beetle (*Tribolium confusum*, Coleoptera: *Tenebrionidae*) and Mediterranean flour moth (*Ephestia kuehniella*, Lepidoptera: *Pyralidae*). Similar results were found also in an experiment reported in [42], where egg, larval, and adult stages of red flour beetle (*Tribolium castaneum*, Coleoptera: *Tenebrionidae*) were treated by NTP. In all flour beetles' stages, 100% mortality can be achieved depending on plasma exposure time and plasma intensity. Optimal effect of plasma with an impact on all stages of *T. castaneum* was observed for 15 minutes.

This review demonstrates that NTP technology is a promising tool for effective decontamination, which offers a wide range of possible applications including inactivation of surface microorganisms and germs of insect pests on cereal grains.

3.2. Surface Property Affection. Together with the surface decontamination, the affection of surface properties should be expected. It is curious that these expected side effects are generally positive.

The NTP treatment caused cracks occurring on wheat grain surface, which led to the improvement of water uptake, subsequently benefiting its germination [43]. After NTP treatment, the mean water uptake was 41% in the control sample and 57% in the exposed one [44]. Similar etching effect, resulting in the improvement of its water absorption capacity, was reported by Li et al. [45]. Also, Rahman et al. [42] stated that the surface of wheat grains was much rougher in exposed grains as compared to that of control ones. Further, the grain coat becomes eroded and chapped after NTP. Similarly, Roy et al. [46] found that NTP treatment caused noticeable morphological changes on seed surface and consequently slightly increased absorption of water. Also, Zahoranová et al. [37] showed that wheat grains treated with NTP took more water than the untreated ones, increasing exposure dose of plasma also increased the water uptake.

The surface hydrophobicity of wheat grains after different applications of NTP was measured by Los et al. [36]. They found that surface hydrophobicity decreased after direct NTP treatment and remained unaffected in the case of indirect treatment. Dobrin et al. [47] also showed interest in wheat grain wettability; they found that the contact angle measured between grain surface and water drop was 92° on the untreated grains, and it decreased to 53° on treated grains. The water absorption was higher in the case of the treated grains as compared with the control samples, too. Likewise, Roy et al. [48] recognized that the surfaces of the treated wheat grains become rough with respect to the control. The changes of surface erosion and roughness may be associated with the enhanced water permeability into the grains. The best water absorption effect (27% more than in control) was found after 12 minutes of NTP treatment. This surface wetting is an important precondition of faster seeds wetting and germination [31], as described in the next section.

3.3. Seed Germination and Seedling Initial Growth. The NTP treatment of grains significantly influences germination and initial growth of plants. The characteristics observed in the experiments included several parameters such as number of germinated grains, germination rate, speed of germination, germination index, grain vitality, length and weight of seedlings, root/shoot (R/S) ratio, and many others. However, the authors usually do not provide their explicit definitions, so we refer to them as they are presented in the original papers.

The germination of many plant species can be improved by NTP treatment. The positive effect of NTP was found on barley (*Hordeum vulgare*, [49]), maize (*Zea mays*, [50]), oat (*Avena sativa*, [51]), rice (*Oryza* sp., [52]), spinach (*Spinacia oleracea* L. Beiouzhizun, [53]), bell pepper (*Capsicum annuum* L. cultivar California Wonder, [54]), and mung bean [55]. On the other hand, the neutral effect was found on oat germination and early growth [43].

Probably, the first researches on wheat grains germination under the effect of different physical factors (ionizing, laser, and NTP) were recorded in the 80s of the last millennium and reported in [56, 57], as mentioned in [51]. Intensity of the initial germination was improved at the expense of accumulation of germ mass; an increase of root length and mass were observed. Under the influence of all physical factors used, field germination increased from 4% to 22% depending on weather conditions.

Šerá et al. [43] tested wheat grains from a private collection, where the grains had been stored for 15 years in dry place before the experiment. Significant difference was found in seed germination at the 4th day of cultivation between a control sample (0%) and the 180 s NTP-treated sample (6%). Above all, the treated grains started to germinate at the 4th day and control grains at the 8th day.

Treatment by cold helium plasma of 80 W could significantly improve germination potential (6.0%) and germination rate (6.7%) of wheat seed. At the seedling stage, plant height (20%), root length (9.0%), and fresh weight (22%) were improved significantly, also the chlorophyll content (9.8%), nitrogen (10.0%), and moisture content (10.0%) were higher than those of the control [58].

Similar results were also reported by Meng et al. [59] using DBD plasma with various surrounding gases (oxygen, air, argon, and nitrogen), and they observed significant increase of germination potential by 24.0, 28.0, and 35.5% after 4 minutes of exposure.

In the work of Guo et al. [44], the germination potential, germination index, and vigor index of wheat grain significantly increased after NTP treatment by 31%, 14%, and 55% over control, respectively. The mean germination rate increased from 88 % in the control sample to 95% in the NTP-treated sample. The improved germination rate and seedling vigor of wheat grains were reported also in [42], where the cumulative germination increased from 70% up to 85% for control and treated samples, respectively. Zahoranová et al. [37] and Zhang et al. [60] consistently found a significant effect of NTP treatment on the germination rate rising by 21% and 8%, respectively.

The germination potential, germination rate, germination index, and vigor index increased by 27%, 9%, 17%, and 47% after 7 minutes of NTP treatment, respectively [45]. The optimal time of treatment seemed to be 4 minutes, when germination potential significantly increased to 77% and the germination rate was enhanced to 95% (in comparisons with 62% and 88% of control samples, respectively). The same authors also presented the hypothesis that the changes in the wheat grain germination characteristics suggested an appropriate DBD plasma treatment dose to promote the wheat grain germination. The similar results presented by Gidea et al. [61] showed that the plasma grain treatment for appropriate time improved the germination rate, speed of germination, and speed of growing in the early stage. However, for high NTP doses, i.e., over optimal treatment time (in this case, 30 min), all the growing properties decreased under untreated. Also in [48], the grains treated for 3 and 6 minutes had the highest germination rate 95–100%. However, the further increments of NTP treatment time caused the decrease of the germination rate. Similarly, Roy et al. [46] reported that germination rate, germination index, and vigor index increased for all used exposition times and atmospheres, with optimal exposure time of 6–9 min.

On the contrary, other works do not report the enhancement of samples properties. In the study of Los et al. [36], short plasma treatment had minimal influence on the germination rate of wheat; however, extending treatment time up to 20 min negatively affected this qualitative parameter. In the experiment described in [38], the grain germination percentage of samples treated for up to 5 min was not affected, but it was decreased after 20 min of NTP treatment.

The effects of plasma treatment on wheat seed germination and seedling growth, together with changes in the surface chemistry and characteristics of the wheat seeds exposed to plasma, were investigated by Los et al. [62]. Treatments of 30–60 s significantly enhanced the germination rate and showed positive effects on seedling growth.

Many observed characteristics of seed germination generally showed better parameters, especially at the first days of the seed germination. Seed germination and initial growth of seedlings are closely related, and many authors tested both at once.

In the experiment performed by Dobrin et al. [47], the total length of the root system per wheat grain was obtained by summing the lengths of individual adventive roots of each grain. The distribution of untreated grains was centered at about 33 cm, while that of the treated grains was centered at 36 cm. NTP treatment of wheat grains resulted in a 10% increase of the mean root length. On the other hand, a very slight increase was obtained in the case of shoot length. Grains treated with NTP for 15 min had substantially heavier roots (1.06 g) than the control samples (0.78 g). Shoot weight was about the same in both cases (0.88 g and 0.89 g). There were considerable differences between R/S ratios: the control sample had 0.88, while the treated sample had 1.2.

Concerning with germination, Gidea et al. [61] reported also the increase of average length of roots and sprouts and vigor index, but after exposure times over 30 min, all measured properties decreased close to or under untreated ones. In the experiments of Roy et al. [48], plant lengths from grains exposed for 3 and 6 min increased to 23 cm and 22 cm, respectively, while the control was 21 cm at the same incubation period. After 20 days of sowing, the longest plant height was 23 cm for the exposed seed, while the control was 19 cm. The same author found similar results in the paper [46], where the plant length and dry weight increased for all used exposition times and atmospheres (air and air/O_2). Maximum increase of plant length was achieved by 11% for 6 or 9 min exposures. The significant increase of shoot length, root length, dry weight, and fresh weight of wheat grains was described also in [44].

Significant effects of plasma treatment on growth parameters of wheat seedlings in dry weight (12% increase) and vigor indexes I and II (28% and 36% increase) were measured [37]. Zhang et al. [60] found that the lengths of the wheat root and shoot were increased effectively by 8.7 cm and 3.3 cm, and the dry weight increased by approximately 10%. Li et al. [45] followed the wheat seedling growth after NTP treatment of grain. They observed that the root length, shoot length, fresh weight, and dry weight of the seedlings increased significantly after different NTP exposures; the exposure optimal for grain growth was probably 7 minutes.

The previously mentioned work of Kordas et al. [35] belongs also in this section: they worked with contaminated wheat grains and did not find any significant differences in shoot lengths and weight of the seedling. On the other hand, they found the rapid decrease in the root length (up to 50%). Similarly, no significant differences were recorded in the previously mentioned experiment on 15-year-old wheat grains [43].

Characteristics of seed germination under laboratory conditions are usually different from real-field data. All environmental influences cannot be controlled in the field, so real germination and development may differ from laboratory results. In the future, it seems necessary to plan experiments for germination tests in the field as well. Two such studies are mentioned below.

3.4. Biochemical Characteristics, Secondary Metabolites, Stimulations, and Stress Reactions. For the sake of completeness, it should be mentioned that NTP influences several biochemical characteristics of grains. However, due to less number of papers, no generalization or conclusion can be made. Some experiments indicate penetration of active

species from NTP through the porous seed coat inside the grain, where they react with plant cells (e.g., [43]). We bring further indirect evidence from experiments on wheat grains.

Amount of secondary metabolites represented by phenolic compounds was studied in wheat seedlings after NTP treatment of the grains [43]. Contents of two phenolic compounds from shoot extract of treated wheat grains were increased to 151% and 165% in comparison with the control sample. The biggest differences of phenolic compound contents were found in the shoot extract than in root extracts.

No alternations in fat, protein, ash, and moisture content of wheat after NTP treatment were found by Rahman et al. [42] and Shahrzad et al. [41]. Guo et al. [44] reported that the active species from NTP penetrated into the caryopses and activated their physiological reactions, resulting in enhancement of soluble protein content by 15% and α-amylase activity by 51%.

According to Tolouie et al. [63], the lipase and lipoxygenase activities are essential for the enhancement of grain shelf life. The result of this work concluded that NTP inactivates these enzymes and increased the shelf life. The lipase and lipoxygenase activity was reduced after 25 min exposure to 25% and 50% of initial extent, respectively. Increasing exposure time and voltage can enhance the inactivation. However, NTP treatment could not permanently inactivate lipase and lipoxygenase. Despite the recovery of enzymes activity during the storage, the enzyme activity of treated samples was much lower than the untreated ones after 30 days of storage at room temperature.

Furthermore, Iranbakhsh et al. [64] reported the NTP-induced expression of heat shock factor A4A, improving the wheat growth and slightly increasing the resistance against salt stress. These results concluded that NTP can enhance the shelf life of wheat grains.

3.5. Wheat Production. Previous results show the possibilities of NTP to improve the properties of wheat grains and seedlings. For general impression, the effect of the final product also should be mentioned. If the NTP should be used to treat cereal grains, it is important that the crop production should be higher or at least the same. However, more field experiments to study the effect of NTP treatment on the farm production are needed.

The effects of the NTP treatment of wheat grains on cereal spikes as a final agricultural product are presented in the two following works. He et al. [65] cultivated three different cultivars of wheat grains treated with NTP for 15 s and sown on the field. The results showed that the spike length and number of grains per spike were improved observably in all tested wheat cultivars. However, the thousand-grain weight and grain protein content increased only by 0.93 g, i.e., by 1%. On the other side, Roy et al. [48] suggested that, beside the increase of germination potential, vigor index, and noteworthy improvement in photosynthetic pigment, the significant increase in the total number of grains per spike, thousand-grain weight, and yield of wheat by 5% also occurred. Moreover, the same authors [46] reported a yield increase by $\sim$20%.

4. Wheat Flour after NTP Treatment

NTP treatment may not be targeted only on cereal grains. Some researchers studied also the effect of NTP treatment of flour and reported several interesting results.

Mahendran [66] confirmed the mortality of adult *Tribolium castaneum* beetle caused by NTP treatment of wheat flour. This mortality was under specific conditions up to 100%; no changes in the color of the flour were observed (see also Section 3.1).

The potential of cold plasma as a tool to modify wheat flour functionality was confirmed by Bahrami et al. [67]. They found that NTP treatment did not affect the concentration of total nonstarch lipids and glycolipids. This treatment, however, reduced total free fatty acids (extracted by *n*-hexane : diethyl ether 1 : 1) and phospholipids. Oxidation markers (hydroperoxide value and head space *n*-hexanal) increased with treatment time and voltage of discharge, which confirmed the acceleration of lipid oxidation. The impact of NTP on free fatty acids was due to a reduction in all major fatty acids; this was more evident in the most oxidatively labile fatty acid, and linolenic acid reduced by 100%. Total proteins were not significantly influenced by NTP treatment, although there was a trend towards higher molecular weight fractions, which indicated protein oxidation, and treated flour did produce thicker dough. However, this work reported no change in the total aerobic bacterial count or total fungi count as a result of NTP treatment.

Structural and functional properties of dough prepared from wheat flour after NTP treatment were studied also by Misra et al. [2]. The elastic and viscous moduli of dough and the dough strength increased after the treatment by optimal NTP dose.

5. Discussion

Based on the available literature, the authors compiled an overview of the results, demonstrating the relationship between NTP and wheat grains. It was difficult to compare the accumulated results because the apparatus and discharges used are not sufficiently described, as are many measured grain characteristics.

NTP is intensively studied in relation to seed modification, above all in relation to the agriculture [29, 32] and food industry [68, 69]. The strategy of using NTP in the food industry is to decontaminate of food products, packaging material processing, functionality modification of food materials, and dissipation of agrochemical residues [4, 29]. Many microorganisms are sensitive to the NTP. So, the indisputable benefit of using NTP is a safe seed without chemical residua, because effective NTP treatment works with clean air, water vapor, or plasma-activated water [70, 71]. Reviewed works clearly demonstrate that the NTP causes effective decontamination of wheat grains from bacteria, fungi, and insect pests with minimal damage of the grains.

In general, lower treatment times will maintain or even improve seed viability. The seeds of many plant species were vital after short time of NTP treatment; they germinate well, e.g., many grains [49–51, 71, 72]. Usually, wheat grains have

good vitality after plasma application. Moreover, due to inactivation of several enzymes, plasma treatment can enhance the wheat grain shelf life. Other works demonstrated not only the enhancement of germination and initial state of growth, albeit they suggest a small increase in the final yield.

The NTP treatment may cause negative effect in some plant species in seed germination, because some plant species are probably more sensitive to NTP than wheat grains, e.g., oat (*Avena sativa*) [43]. Cultivars of both the hemp seeds (*Cannabis sativa*) [73] and poppy seeds (*Papaver somniferum*) [74] were affected by NTP in a different way. Some cultivars were positively affected, while others were not. The type of plasma apparatus/discharge and the parameters of the plasma treatment are crucial too. Significant differences were found in the germination and early growth of buckwheat seeds (*Fagopyrum esculentum*) [75] and hemp seeds [73] when different apparatus were used.

Concerning the treated wheat flour, plasma may kill the unwanted adult beetles and affects its functional properties, producing the qualitatively better dough. So far, there are no attempts with other types of flour to obtain the necessary comparison.

6. Conclusion

Plasma decontamination of wheat grains has a great potential to be applied in practice. Reviewed works clearly demonstrate that the plasma causes effective decontamination from bacteria, fungi, and insect pests with minimal damage of grains. Moreover, due to inactivation of several enzymes, plasma treatment can enhance the grain shelf life. Other works demonstrated not only the enhancement of germination and initial state of growth, albeit they suggested a small increase in the final yield. Concerning the treated flour, plasma may kill the adult beetles and affects its functional properties, producing the qualitatively better dough.

It can be stated that NTP treatment of wheat grains has a great potential to be applied in the food industry. The NTP can be used in wheat grain surface disinfection, grain germination and vitality improving, and wheat flour modification and disinfection. The paper brings structured overview of existing knowledge in this area.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Effect of Radio Frequency Cold Plasma Treatment on Intermediate Wheatgrass (*Thinopyrum intermedium*) Flour and Dough Properties in Comparison to Hard and Soft Wheat (*Triticum aestivum* L.)

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Cold plasma is an emerging technology to improve microbiological safety as well as functionality of foods. This study compared the effect of radio frequency cold plasma on flour and dough properties of three members of the *Triticeae* tribe, soft as well as hard wheat (*Triticum aestivum* L.) and intermediate wheatgrass (*Thinopyrum intermedium*, IWG). These three flour types differ in their protein content and composition and were evaluated for their solubility, solvent retention capacity, starch damage, GlutoPeak and Farinograph profiles, and protein secondary structures. Plasma treatment resulted in dehydration of flours but did not change protein content or solubility. Farinograph water absorption increased for all flours after plasma treatment (from 56.5–61.1 before to 71.0–81.6%) and coincided with higher solvent retention capacity for water and sodium carbonate. Plasma treatment under our conditions was found to cause starch damage to the extent of 3.46–6.62% in all samples, explaining the higher solvent retention capacity for sodium carbonate. However, Farinograph properties were changed differently in each flour type: dough development time and stability time decreased for hard wheat and increased for soft wheat but remained unchanged in intermediate wheatgrass. GlutoPeak parameters were also affected differently: peak torque for intermediate wheatgrass increased from 32 to 39.5 GlutoPeak units but was not different for the other two flours. Soft wheat did not always aggregate after plasma treatment, i.e., did not aggregate within the measurement time. It was also the only flour where protein secondary structures were changed after plasma treatment, exhibiting an increase from 15.2 to 27.9% in β-turns and a decrease from 59.4 to 47.9% in β-sheets. While this could be indicative of a better hydrated gluten network, plasma-treated soft wheat was the only flour where viscoelastic properties were changed and extensibility decreased. Further research is warranted to elucidate molecular changes underlying these effects.

1. Introduction

Nonthermal plasma offers a multitude of application options for food scientists. Aside from increasing microbiological safety, it may also affect the functionality of food constituents such as starch and proteins [1]. For instance, nonthermal plasma treatment of starch has been reported to result in cross-linking [2] but also cleavage of glycosidic bonds [3]. As for the effect on protein, studies have reported changes in solubility [4, 5], secondary structure distribution, as well as other functional parameters such as emulsification properties [1]. Advantages of using nonthermal plasma include low losses of nutrients or sensory properties and its suitability for treatment of heat-sensitive materials [1]. Cold plasma has been proposed as a nonthermal treatment of flours to enhance functionality in wheat [6]. Previous research has reported oxidative changes in flour, which may modify dough properties [7]. Flours contain numerous components that may be affected by plasma treatment, most importantly the gluten-forming properties, starch, nonstarch polysaccharides, and lipids [8]. Studies often use different conditions of plasma treatment, and thus

a systematic evaluation of the effect of nonthermal plasma on main flour constituents, in dependence of treatment conditions such as carrier gas, is warranted. Misra et al. [9] reported that atmospheric pressure cold plasma treatment in the presence of air affected functional and structural parameters of soft and hard wheat flours. This change was related to proteins exhibiting a more ordered structure, increased dough strength, and modulation of the mixing behavior and viscoelastic properties.

Evaluating the effect of nonthermal plasma on different types of cereal flours is of interest due to their different suitability for certain products, e.g., hard wheat (HRW) for bread, soft wheat (SW) for cookies, crackers, cakes, or other products [10]. We have previously reported on chemical and functional characteristics of the intermediate wheatgrass (*Thinopyrum intermedium*, IWG) [11–13], a perennial crop with environmental benefits such as reduced nitrate leaching [14]. One limitation to its use as stand-alone flour is that it has poor gas-holding capability, due to being deficient in high-molecular-weight glutenins [12, 13].

Our overall aim for this study was to investigate the effect of radio frequency cold plasma treatment on flour and dough properties and to evaluate how protein properties, in particular gluten network formation in dough, were affected. Three *Triticum* genus members with different gluten-forming properties were contrasted: hard wheat usually has better viscoelastic properties and forms stronger gluten networks than SW [15]. Because bran to endosperm ratios are higher compared to annual crops such as hard wheat [16], IWG's total protein and insoluble dietary fiber contents are higher than annual crops, but its high dietary fiber and low glutenin contents negatively impact viscoelastic properties [11, 12].

The properties of dough systems as well as quality of products, especially when leavened, crucially depend on the formation of a strong gluten network [15]. We therefore evaluated changes in protein characteristics to assess the effect of nonthermal plasma on flour functionality.

2. Materials and Methods

2.1. Materials. Hard red wheat was graciously provided by Grain Millers Inc. (Eden Prairie, MN). Intermediate wheatgrass was grown in Rosemount, Minnesota, US, and obtained through the Department of Agronomy and Plant Genetics, University of Minnesota. Commercial soft wheat provided by Horizon Milling LLC (Mankato, MN, USA) was used. Samples were milled with a Quadrumat Junior mill (C. W. Brabender, South Hackensack, NJ, USA). Chemical reagents employed were of reagent grade or higher.

2.2. Radio Frequency Cold Plasma Treatment. About 10 g flour were weighed into 2 glass Petri dishes with inner diameters of 15 cm, spread out in thin layers (≈2 mm) as proposed previously [17], and subjected to radio frequency-generated cold plasma treatment based on conditions reported by Spencer and Gallimore [18] in a Plasma Etch PE75 (Plasma Etch, Carson City, NV, USA) operating at 120 W. Argon and carbon dioxide were used at flow rates of 10 and 25 cm^3/min, respectively. A cooling unit set to 25°C was used. Cycles began at a pressure of 0.6 atm. Samples were treated for 1 hour in two 30-minute cycles and stirred in between. The stirring step was implemented to achieve a more even treatment throughout the flour layer in the Petri dish.

2.3. Protein Content and Solubility. Protein content was quantified by Dumas procedure according to AACCI method 46-30.01 [19] on a TruSpec N (Leco 165 Corporation, St. Joseph, MI) calibrated with glycine. Protein solubility was assessed after sample extraction as described by Marengo et al. [20] except that sample amounts and extraction volume were downscaled by a factor of 10 to 50 mg and 1 mL, respectively.

2.4. Solvent Retention Capacity and Starch Damage. Solvent retention capacity profiles of each sample were assessed in duplicate through AACCI method 56-11.02 [19]. Starch damage was quantified with a Megazyme (Wicklow, Ireland) starch damage assay kit based on AACCI method 76-31.01 [19].

2.5. Farinograph Evaluation and Dough Extensibility and Resistance to Extension. A Brabender Farinograph (C. W. Brabender) equipped with a 50 g bowl was used to assess flours according to AACCI standard method 54-21.01 [19]. The parameters obtained included the time required to form an optimum dough (dough development time, DDT), the time for which this dough was stable (dough stability, DS), and the optimum amount of water required to form such a dough (Farinograph water absorption, FWA).

2.6. Assessment of Dough Extensibility and Resistance to Extension. For assessments of extensibility (mm) and resistance to extension (g), dough was prepared in a 10 g Farinograph bowl, sampled at the DDT according to the method described by Banjade et al. [16]. A TA.XT-Plus Texture Analyzer (Texture Technologies, Hamilton, MA) equipped with a Kieffer dough and gluten extensibility rig was used with Texture Exponent 32 version 6.0.6.0 software (Texture Technologies, Corp. Scarsdale, NY, USA). A total of 7 dough strips were tested from each dough replicate.

2.7. Protein Secondary Structures. Dough was prepared in the Farinograph, using the conditions described in Section 2.5, and sampled at the dough development time, as assessed by pretrials. Spectra of the dough were then recorded at least in triplicate on a Bruker Tensor 37ATR-FTIR spectrophotometer (Bruker Optics, Inc., Billerica, MA, USA) equipped with a horizontal multireflectance zinc selenide crystal accessory as described by Marti et al. [21], using OPUS 7.0 software. Protein secondary structures were calculated using second-derivative spectra of amide I regions (1600–1700 cm^{-1}), assigning 1620–1644 cm^{-1} as β-sheets,

1644–1652 cm^{-1} as random structures, 1652–1660 cm^{-1} as α-helix, and 1660–1685 cm^{-1} as β-turns.

2.8. GlutoPeak Analysis. The aggregation properties of flours before and after plasma treatment were assessed in duplicate on a Brabender GlutoPeak (C. W. Brabender) based on the method reported by Chandi and Seetharaman [22]. Flour moisture contents were measured on an Ohaus MB45 infrared balance on the day of the analysis. The time to reach peak maximum time (PMT, in s), the maximum torque (MT, in GlutoPeak units, GPU), and the aggregation energy (AE, in GPU) were determined using Brabender GlutoPeak v. 2.1.2 software.

2.9. Statistical Analysis. One-way analysis of variance (ANOVA) was performed in R (version 3.1.0) [23], two-way ANOVAs (with flour type and plasma treatment as factors), and paired *t*-tests (to differentiate flours before and after plasma treatment) in Excel (Microsoft, Redmond, VA). Differences among means were calculated with Tukey's honestly significant difference test, at $\alpha = 0.05$.

3. Results and Discussion

3.1. Protein Solubility. Protein solubility was evaluated in three media, i.e., buffer containing a low concentration of sodium chloride, the same buffer additionally containing 8 M urea, and buffer with 8 M urea and disulfide cleaving agent dithiothreitol (Figure 1). While the type of cereal and the solvent both had a significant effect on the solubility, the plasma treatment did not. Partly, our results are in contrast to Marti et al. [11], who reported that IWG flour had higher solubility in phosphate buffer than hard red wheat, in line with its reported higher contents of albumins and globulins [12]. These differences may be related to different flour samples used in our studies. In general, changes in protein solubility in buffers with different additives indicate how different protein fractions respond to a given treatment.

Plasma treatment did not significantly enhance or decrease protein solubility in any of the media used. Changes in protein solubility over processing can indicate if protein interaction and aggregation are affected by the treatment [20]. Adding dithiothreitol and/or urea solubilizes proteins that are either insoluble in water or dilute salt solutions or proteins that originally were soluble but became insoluble over the course of the treatment step [24]. Based on our data, plasma treatment did not alter protein interactions of any protein fraction, in any of the flour types, in a way that affected solubility.

3.2. Solvent Retention Capacity and Starch Damage. Solvent retention capacity (SRC) assessed flour swelling in four solvents which differ in their compatibility to the three main polymers, i.e., 5% lactic acid (La) to assess gluten swelling, 5% sodium carbonate (SC) to assess swelling due to starch damage, 50% sucrose (Su) to assess arabinoxylan-mediated swelling, and swelling in pure water (W), which is influenced by all three components [25, 26]. A change in the ability of a flour to swell in different solvents would indicate that the treatment affected the polymer targeted by the solvent. SRC was implemented to see if nonprotein polymers were being affected by plasma treatment. Before and after plasma treatment, HRW had the highest La-SRC, Su-SRC, and W-SRC (Table 1). SW had significantly higher La-SRC before and after plasma treatment than IWG, reflecting poor ability of IWG to form gluten networks [12] and lack of change from treatment. Overall, SRC values for HRW and SW were in the range of several previous studies [27–29], whereas to the best of our knowledge, no prior reports for IWG SRC have been published. The most notable observation in our sample set was that upon plasma treatment, the W-SRC of HRW and SW significantly increased (by 17 and 27%, respectively), whereas the change was not significant ($p = 0.056$) for IWG. In all three flours, the changes in W-SRC coincided with significantly ($p < 0.05$) increased SC-SRC; however, the flours were affected to a different degree. In untreated flours, the ranking for SC-SRC was IWG > HRW > SW, whereas after plasma treatment it changed to HRW > IWG > SW. While HRW and SW experienced an increase of ca. 30% for SC-SRC, it was only ca. 14% for IWG. In contrast, La-SRC and Su-SRC were not altered by plasma treatment, suggesting that gluten-forming proteins and arabinoxylans were not modified by plasma treatment, or at least not modified in a way to affect swelling behavior. Overall, the SRC results suggested that plasma treatment increased the level of starch damage of flours and that this also affected water absorption. Thus, starch damage levels were analyzed in addition to SRC measurements. While plasma treatment resulted in significantly ($p < 0.05$) higher starch damage levels in SW (from 3.34 in SW to 3.52% in SW + P) and HRW (from 6.03% in HRW to 6.62% in HRW + P), IWG's levels slightly, but significantly ($p < 0.05$) decreased (from 3.59% in IWG to 3.46% in IWG + P). Therefore, the influence of plasma treatment on parameters indicative for dough functionality may be higher for SW and HRW.

3.3. Protein Secondary Structures. The ratio of proteins folded into a certain conformational type has been demonstrated to reflect protein network formation in dough systems [30]. The viscoelastic properties of dough systems are influenced by β-turns and β-sheets [31], with the former being indicative of hydrated protein regions (referred to as "loops"), and the latter denoting regions of protein-protein interactions (referred to as "trains"). In our samples, the flour type significantly affected protein secondary structures: in hard and soft wheat, β-sheets represented the main conformational arrangement within the proteins, followed by β-turns (Table 2). Before plasma treatment, soft wheat flour had a significantly lower content of β-turns than the other two flours. IWG had a significantly lower content of β-sheets than the other flours before and after plasma treatment, in agreement with data from a study that compared whole IWG to hard red wheat [11]. No differences were observed among samples in random structures or

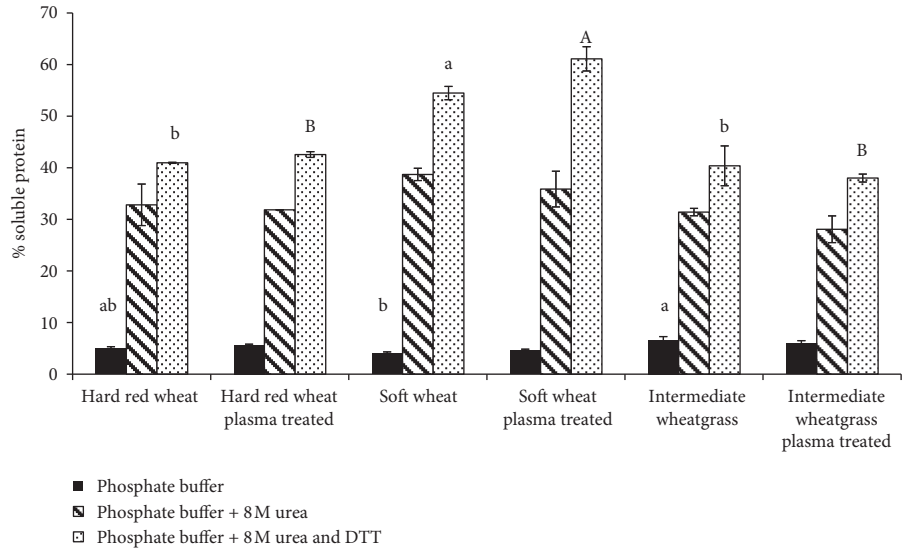

Figure 1: Protein solubility of hard red wheat, soft wheat, and intermediate wheatgrass flour in three solvents (pH 7 phosphate buffer containing 0.1 M sodium chloride, the same buffer with additional 8 M urea, and buffer with 8 M urea as well as 0.01 M dithiothreitol (DTT)). Error bars represent standard deviations, different lowercase letters represent differences in the solubility in the same solvent among samples before plasma treatment, and uppercase letters represent differences after plasma treatment, assessed via Tukey's HSD test ($p < 0.05$). Solubility in pH 7 phosphate buffer did not significantly differ in plasma-treated samples, and solubility in pH 7 phosphate buffer with 8 M urea did not significantly differ among any samples.

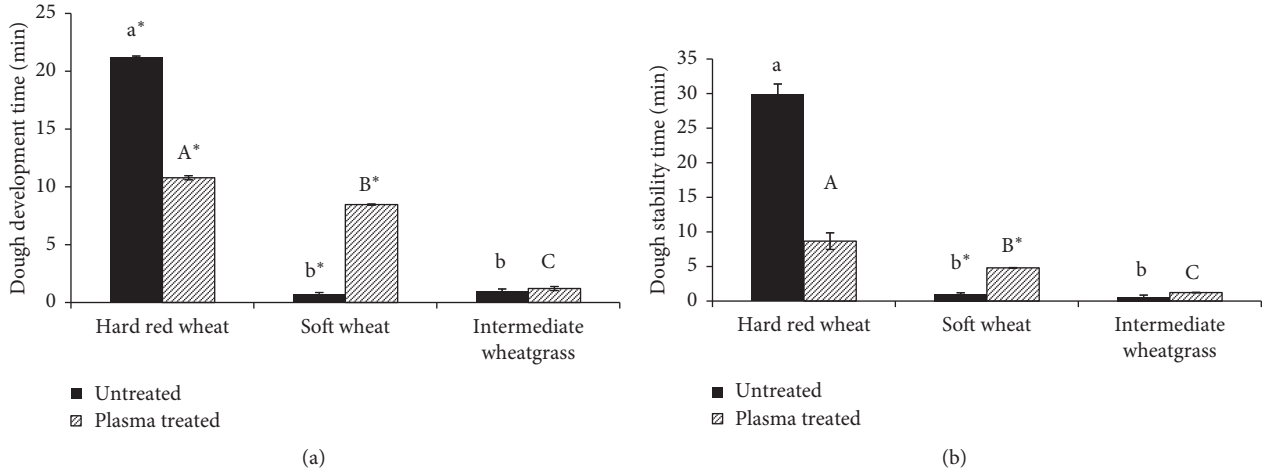

Figure 2: Dough development time (a) and stability (b) of hard red wheat, soft wheat, and intermediate wheatgrass flour. Error bars represent standard deviations ($n = 2$), and different lowercase and uppercase letters represent differences ($p < 0.05$) between flour types before and after plasma treatment (assessed by Tukey's HSD test), respectively. Asterisks represent differences ($p < 0.05$) within the same flour type due to plasma treatment (assessed by a paired t-test).

α-helices. Plasma treatment affected the samples differently: most notably, in soft wheat the proportion of β-turns significantly ($p < 0.05$) increased, while the proportion of β-sheets significantly decreased ($p < 0.05$). No significant changes were observed in IWG or hard wheat.

While Issarny et al. [29] did not find differences in protein conformations in dough from hard and soft Canadian wheat flour, Katyal et al. [32] reported that dough from extraordinarily soft wheat had fewer proteins with intermolecular β-sheet conformation than hard wheat, which could indicate fewer interactions among proteins. In our samples, the decrease in β-sheets observed for soft wheat due to plasma treatment could be indicative of lower ability for protein aggregation, which could impair the formation of networks capable of gas holding [33].

3.4. Farinograph and Viscoelastic Properties of Dough. Before plasma treatment, hard wheat had significantly ($p < 0.01$) higher DDT (Figure 2(a)) and DST (Figure 2(b)) than the other two flours, which did not significantly differ from each other. In general, flours with a stronger gluten

Table 1: Solvent retention capacity of flour types before and after plasma treatment.

Solvent	Hard red wheat	Intermediate wheatgrass	Soft wheat
Before plasma treatment			
Lactic acid	126.3^{a}	78.7^{c}	86.4^{b}
5% sodium carbonate	91.1^{b*}	98.1^{a*}	80.6^{c*}
50% sucrose	114.3^{a}	107.2^{b}	103.1^{c}
Water	79.1^{a*}	73.3^{b}	71.3^{b*}
After plasma treatment			
Lactic acid	121.3^{A}	79.2^{C}	88.6^{B}
5% sodium carbonate	120.0^{A*}	111.8^{B*}	104.7^{C*}
50% sucrose	140.5^{A}	115.3^{B}	108.7^{B}
Water	92.5^{A*}	84.1^{B}	90.9^{AB*}

Different letters indicate differences among values across rows, assessed via Tukey's HSD test ($p < 0.05$), i.e., between flour types for the same solvent. Lowercase letters were used to signify differences before plasma treatment and uppercase letters to distinguish flour types after plasma treatment. Asterisks denote significant differences (paired t-test, $p < 0.05$) in the proportion of a secondary structure type between untreated and plasma-treated flours of the same type.

Table 2: Proportional contribution of different secondary structures to overall protein secondary structure in hard red wheat, soft wheat, and intermediate wheatgrass before and after plasma treatment.

Protein secondary structure	Hard red wheat	Intermediate wheatgrass	Soft wheat
Before plasma treatment			
β-turns	29.8^{a}	40.4^{a}	15.2^{b*}
α-helices	7.4	16.0	6.0
Random	19.3	11.0	19.5
β-sheets	43.6^{b}	30.2^{b}	59.4^{a*}
After plasma treatment			
β-turns	29.9	39.1	27.9*
α-helices	3.7	9.6	8.8
Random	18.1	16.5	15.4
β-sheets	49.2^{A}	34.7^{B}	47.9^{A*}

No significant differences ($p < 0.05$) were detected among α-helices and random structures. Different letters represent differences among values across rows, assessed via Tukey's HSD test ($p < 0.05$); lowercase letters represent differences among flours before plasma treatment, and uppercase letters represent differences among flours after plasma treatment. Asterisks denote significant differences (paired t-test, $p < 0.05$) in the proportion of a secondary structure type between untreated and plasma-treated flours of the same type, i.e., differences across columns.

network are characterized by higher DDT and DST [34]. Plasma treatment affected the flours differently; it significantly ($p < 0.05$) decreased DDT for HRW and increased it for SW ($p < 0.01$) but did not affect it in IWG. While plasma-treated hard wheat flour still had higher DDT and DST values than the other two flours, plasma-treated soft wheat had significantly ($p < 0.05$) higher values for these parameters than IWG. Two-way ANOVAs for DDT, DST, and water absorption showed the same trend: there was a significant effect of sample type, plasma treatment, and their interaction on the dough properties. The FWA was significantly increased by plasma treatment (data not shown), presumably due to the higher starch damage in samples, in line with previous studies [35].

Previously, hard and soft wheat flour mixing properties have been reported to be affected by plasma treatment in a way that suggests formation of stronger dough systems, possibly due to disulfide linking of proteins when air was used during the treatment [9]. Bahrami et al. [7] reported an increase in high-molecular-weight proteins upon plasma treatment.

The extensibility and the resistance to extension were significantly higher for hard wheat than for SW and IWG before and after plasma treatment (Figure 3), signifying a better viscoelastic gluten network than the other two flours. In comparison to other studies, SW had lower resistance to extension, and extensibility [36]. Moreover, it was the only flour where extensibility decreased as a result of plasma treatment. Starch damage has previously been reported to decrease extensibility and could thus have been the reason for this change [35].

3.5. Protein Aggregation in the GlutoPeak. Before and after plasma treatment, HRW had significantly ($p < 0.01$) higher MT than the other two flours, which did not significantly differ from each other (Figure 4(a)). PMT (Figure 4(b)) was significantly ($p < 0.01$) affected by flour type, plasma treatment, and their interaction, while MT was only significantly ($p < 0.01$) affected by flour type and flour type × plasma treatment interactions, but not by plasma treatment. The MT and PMT of soft and hard wheat before plasma were in line with previous reports [22] that also had observed higher MT for HRW than for SW [36]. Protein aggregation did not vary before and after plasma treatment for HRW as its PMT and MT were not significantly different due to plasma treatment. HRW's AE was not significantly

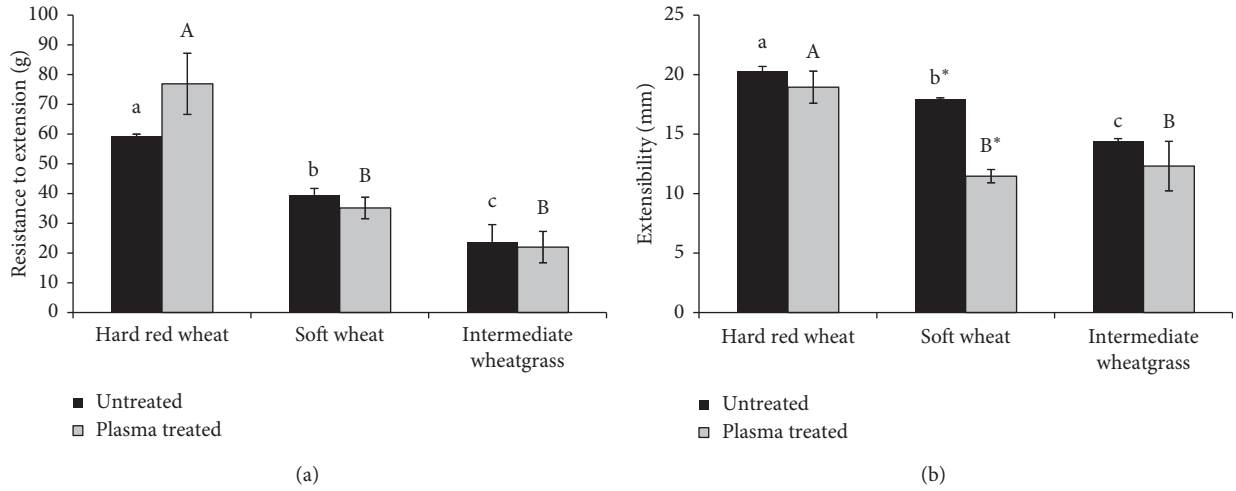

Figure 3: Extensibility and resistance to extension of dough ($n = 2$) prepared from hard red wheat, soft wheat, and intermediate wheatgrass flour before and after plasma treatment.

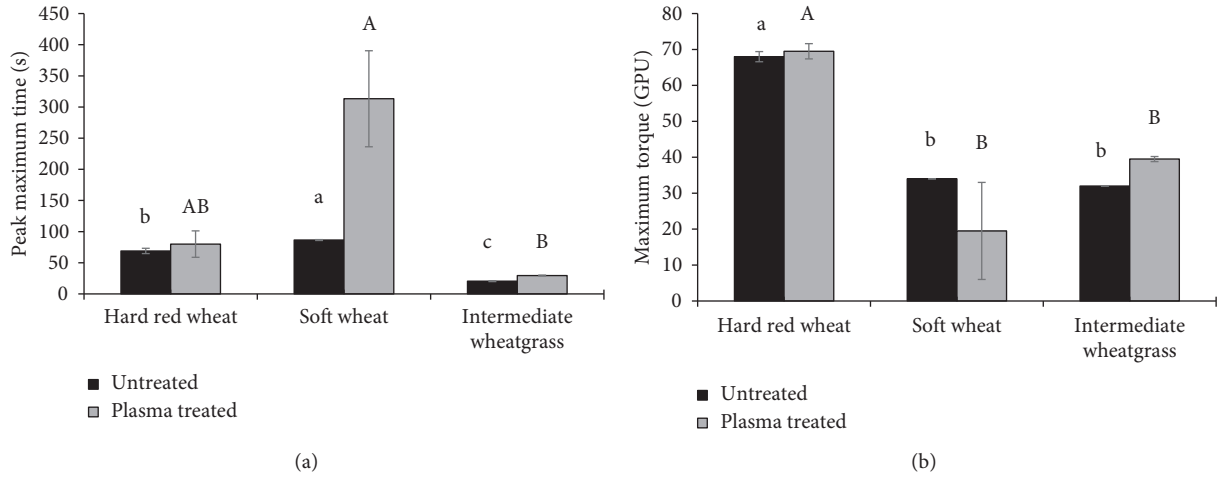

Figure 4: Peak maximum time (PMT) and maximum torque (MT) of hard red wheat, soft wheat, and intermediate wheatgrass flour before and after plasma treatment.

affected by plasma treatment and highest of all flours. In contrast, for IWG, AE significantly increased ($p < 0.05$) after plasma treatment from 678.0 ± 3.6 to 992.9 ± 26. Remarkably, after plasma treatment, SW flour exhibited no aggregation within the allotted time frame of the experiment. Thus, SW was uniquely affected by plasma treatment: while all flours exhibited higher SRC for water and SC (Table 1) and Farinograph water absorption, it was the only sample where this led to inhibition of gluten aggregation. Overall, plasma treatment under our experimental conditions had a negative influence on its properties. Higher levels of starch damage and water absorption are detrimental for the quality of products typically made with soft wheat, such as cookies [37, 38].

4. Conclusions

Dough rheology, secondary structure, and protein aggregation measurements showed that each flour had a different response to plasma treatment. With plasma treatment, SW had a longer period of stability in the Farinograph, no aggregation in the GlutoPeak, and an increase in β-turns at the expense of β-sheets. HRW had a shorter period of stability but no difference in protein aggregation or secondary structure as a result of plasma treatment. IWG had no change in dough stability or secondary structure, but it did show an increase in protein aggregation after plasma treatment. SW and HRW experienced a significant increase in starch damage, and all flours had higher water absorption. The different effects of plasma treatment on flour types present questions for future research to elucidate molecular mechanisms for these changes. Moreover, the functionality of the plasma-treated flours in food products needs to be evaluated.

Data Availability

All data are presented in figures and tables.

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Article

Effects of Multihollow Surface Dielectric Barrier Discharge Plasma on Chemical and Antioxidant Properties of Peanut

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An experiment was conducted to investigate the effects of atmospheric pressure plasma generated by multihollow surface dielectric barrier discharge on chemical and antioxidant properties of peanut. Multihollow surface dielectric barrier discharge is a novel plasma device applicable in food industry applications due to the capacity of the generated plasma to treat the surface of food without changing the quality. Peanut seeds were exposed to the multihollow plasma for different plasma power (10–40 W), air flow rate (0.5–20 l/min), and time (1–15 min). The fatty acid profile, peroxide value, acid value, moisture content, total polyphenols, and antioxidant activity were evaluated during cold plasma treatment. The result revealed that, due to the variation plasma power, treatment time and air flow rate caused a decrease in unsaturated fatty acid and moisture content and increased saturated fatty acids, peroxide value, acid value, and total polyphenols of the peanut.

1. Introduction

Peanuts (*Arachis hypogaea* L.) are a globally important oilseed valued as a source of high-quality cooking oil, crude protein, crude fat, crude fiber, water, ash, total sugar, amino acids, fatty acids, vitamins, minerals, phytosterol, resveratrol, squalene, and other antinutritional factors [1] and appreciated worldwide as an affordable, flavorful, serving as a primary ingredient for peanut butter, confections, and nutritional bars, among other finished products. It is widely used as an economic food enhancement to counter malnutrition owing to its high nutritional value [2]. However, the aforementioned characteristics led the peanut to become sensitive to molds contamination, in the whole supply chains [3] and other biotic and abiotic stresses constrain production and use of peanut [4, 5]. Different microorganisms infect peanuts and cause spoilage, leading to the production of toxic metabolites [6–8]. Various methods have been applied to decontaminate the growth of molds in peanut such as conventional and nonthermal treatments, but none of these methods offers a complete control of toxigenic molds.

A lot of nonthermal technologies have been investigated and applied in food industries to assure and improve the quality of the food. The use of nonthermal surface decontamination processes and surface treatment is desirable for a variety of food products, in particular for those in which it is important to heat sensitive agricultural products. Among those nonthermal technologies, plasma is one of the latest green technologies used now a days around the world for various applications [9, 10].

According to Fridman et al. [11], plasma is often referred to as the fourth state of matter, comprised of several excited atomic, molecular, ionic, and radical species, coexisting with numerous reactive species, including electrons, positive and negative ions, free radicals, gas atoms, molecules in the ground or excited state, and quanta of electromagnetic radiation (UV photons and visible light). Plasma can be

generated using any kind of energy which can ionize the gases, such as electrical, thermal, optical, and radioactive and X-ray electromagnetic radiation. However, electric or electromagnetic fields are widely used for cold plasma generation [12]. Plasma can be generated at low or high pressure but plasma generated at atmospheric pressure is of interest to the food industry because it does not require extreme process conditions [13]. Cold Plasma is also known as nonequilibrium plasma, because of its low gas temperature of <70°C, because the applied energy leads to an elastic collision of the gas particles, atoms, and electrons. The gas particles are less energetic than the electrons in the discharge where the heavy particles have kinetic temperatures close to ambient because the transfer of kinetic energy to other particles in such a way that the cooling of the uncharged particles and neutral ions is more rapid than the energy transfer from the electrons [11, 14, 15].

Cold plasma is a better alternative to other existing surface decontamination methods due to operation at atmospheric pressure, low-temperature, long operative duration, and economical and simple systems [16], and it is a novel and green food preservation technology and has only been applied at very small scales [17]. This technology is gradually finding acceptance among food researchers for the surface sterilization but the effect of cold plasma on the sensitive constituents of foods mainly lipids, vitamins, and bioactive compounds, and the physical quality of the product being treated not addressed [13].

Atmospheric cold plasma surface treatment process offers novel food preservation properties and has been tested with different plasma setup and gas sources in different cereal grains [8, 18], peanuts [6, 8, 19, 20], dairy [21], fruits and vegetables [22], meat [23–25], and spices [26, 27] but none of them have investigated the synergetic effect of cold plasma operating conditions (plasma power, air flow rate, and treatment time) on the quality of the treated food product.

Numerous researches have been done to investigate the effects of plasma on food constituents, and various chemical reactions are induced by plasma, but there has been speculation on the free radical formation when fatty foods and with high antioxidant and polyphenol compounds are exposed to plasma energy. Cold plasma can generate reactive and free radical species, and these species that have strong oxidation capacities. Treating high lipid-containing materials with cold plasma could lead to lipid and consequently to development of off-flavor and off-odor and in loss of natural antioxidants and caused the formation of many volatiles related to lipid oxidation [24]. Peanut contains the high percentage of mono- and polyunsaturated fatty acids and the low percentage of saturated fatty acids [1].

Little information is available about the synergistic influence of cold plasma operation conditions (plasma power, air flow rate, and treatment time) peroxide value, acid value, fatty acid profile, antioxidant activity, total polyphenols, and moisture contents of peanuts. Therefore, the current objective was to study the influence of multihollow surface dielectric barrier discharge plasma operating conditions on chemical and antioxidant properties of peanut.

2. Experimental Details

2.1. Chemicals and Samples. *n*-Hexane, methanol, 95% ethanol, potassium hydroxide, sodium thiosulphate, potassium iodide, chloroform, glacial acetic acid, 2,2-diphenyl-1-picryhydrzyl radical (DPPH), Folin–Ciocalteu, sodium carbonate, starch, and gallic acid. A domestic, commercial peanut (Roba variety) was obtained from the Were Research Center, Oromia, Ethiopia.

2.2. Plasma Treatment of Peanuts. Plasma surface modification of peanuts was carried out using a special reactor for plasma treatment of small peanut samples. The rector was based on commercial coplanar-type multihollow surface dielectric barrier discharge unit for which the detail characteristics and properties were reported by [28]. A schematic draw of the experimental setup is shown in Figure 1. Multihollow surface dielectric barrier discharge plasma (MSDBD) is composed of two planar metal electrodes at distance 0.5 mm, both embedded in alumina ceramic. The entire surface is perforated by creating the 18×18.9 mm ($\sim 3.4\ cm^2$). Multihollow surface DBD plasma was generated by a sinusoidal alternate current (~27 kHz), high-voltage (10 kV) power source. An MSDBD unit was embedded in a feed chamber enabling the plasma generation at a certain flow of plasma forming gas.

Plasma treatment of peanuts was done at varied treatment conditions. Total input power, monitored by a commercial wattmeter, was 10–40 W. The flow rate of ambient air with humidity 20–30% was controlled by the thermal mass flow controller RED-Y in the range (0.5–20 L/min). The treatment time was varied from 1–15 min, based on rotatable central composite design. IR thermometer, FLUKE 62 MAX, 3 M DROP water/dust resistance, IP54, with the temperature range −30 to 500°C was used to measure the temperature of the ceramic during the experiment. Six peanuts were treated by plasma in one batch. The peanuts were mechanically moved and turned around by inert plastic rod during the plasma treatment to provide a homogeneous surface treatment of peanuts. The samples were taken from the plasma field after treatment, and the treated peanuts were cooled to room temperature, packed in polyethylene bags, and kept at 4°C for further analysis.

2.3. Sample Preparation for Extraction. The cold plasma-treated and the untreated peanut seeds were milled (High-Speed Universal Disintegrator (FW100) Grinder, China) with a speed of rotating knife (2400 rpm) and passed through a mesh size 16 sieve to obtain identically sized particles and then was retained in a sealed bag in a refrigerator (1-2°C) until use. Milled peanut seed particle size is important to facilitate analyses of mass transfer during the extraction of oil and antioxidant.

2.4. Extraction Methods. The extraction was performed in duplicate, with solvent, *n*-hexane (99% purity). An automated Soxhlet set (The Soxhlet extractor SXT-06,

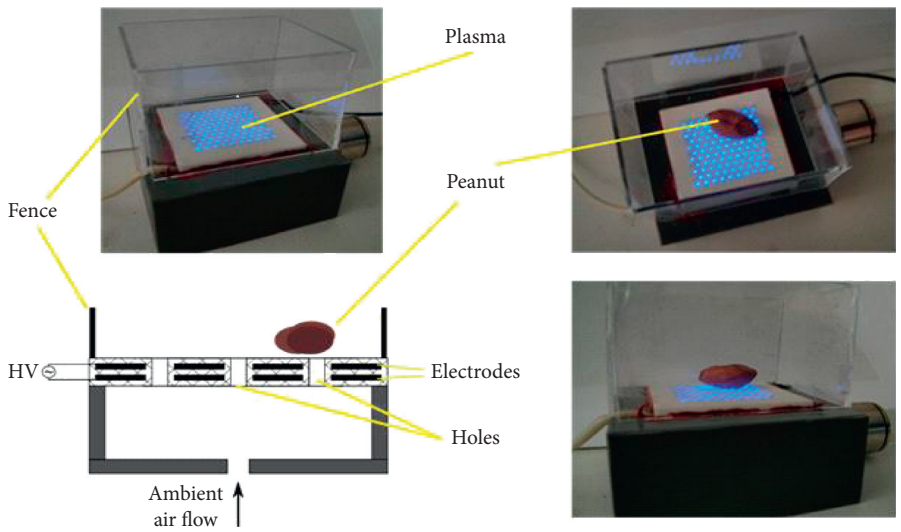

Figure 1: Multihollow surface DBD electrode setup.

Shaanxi, China) was used to extract peanut oil. To achieve this, 5 g of sample was packed in a cartridge placed inside a 250 mL extractor device. The sample was extracted for 8 h. Then extra solvent form sample oil was removed by the rotary vacuum evaporator. The extracted solvent was stored in a brown bottle in the refrigerator for further analysis.

2.5. Extraction of Antioxidant Components. The plasma-treated peanut seeds were defatted first with *n*-hexane (10% w/v) using a Soxhlet extraction unit for 8 h. The defatted samples were then air-dried and extracted with methanol (100 mL) using an incubator shaker (Thermo Shaker Incubator, Model, THZ-103B, China). All suspensions were then filtered through a Whatman No. 1 filter paper, and the residues re-extracted twice, each time with an additional 100 mL of the same solvent. The filtrates were combined and the solvent evaporated under reduced pressure using a rotary evaporator (Eyela, Model N-1000) at 40°C. The methanolic extracts were used for the determination of total polyphenol and antioxidant activity.

2.5.1. Extraction of Peanut for Analysis of the Antioxidant and Polyphenols. Samples were extracted based on the procedures used by Bishi, et al. [29]. Briefly, five grams of dried groundnut powder was extracted by stirring with 50 ml of methanol at 25°C at 150 rpm for 24 h using the temperature shaker incubator (ZHWY-103B) and then filtered through Whatman No. 4 paper. The residue was then extracted two wises with the addition of 50 mL methanol as the above procedure. The combined methanol extracts were evaporated at 40°C to dryness using a rotary evaporator (Stuart R3300). The crude extracts were weighed to calculate the yield and redissolved in methanol at the concentration of 30 mg/ml and stored in a refrigerator (−4°C) until used for further work.

2.5.2. Measurement of Antioxidant Activities and Total Polyphenol

(1) Total Polyphenols Contents (TPC) Determination. A modified Folin–Ciocalteu procedure as described by [30] was used for the determination of total polyphenol contents. Samples (0.1 mL) were mixed with 1.0 mL of the Folin–Ciocalteu reagent (previously diluted with distilled water 1 : 10 v/v), and the reaction was terminated using 1 mL of 7.5% sodium carbonate. The mixture was vortexed for 15 sec for color development. After 30 min incubation at room temperature (28 ± 1°C), the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (PerkinElmer Lamda 950 UV/Vis/NIR). The standard curve was prepared using gallic acid standard solutions of known concentrations, a linear calibration graph (Figure 2) was constructed with gallic acid concentrations of 20, 50, 100, 150, 200, and 250 μg/mL, and the results were expressed as mg gallic acid equivalent/100 g sample:

$$\text{TPC} = \frac{\text{C} \times \text{V}}{\text{M}}, \quad (1)$$

where TPC = total polyphenol content(mg/gm); C = concentration of gallic acid (mg/mL); V = volume of extract in assay (mL), and M = mass of pure plant methanolic extract (gm).

2.5.3. Free Radical Scavenging Assay (DPPH). The effect of methanol extracts on DPPH radical was estimated according to Win et al. [31]. A 0.004% freshly prepared solution of DPPH radical solution in methanol was prepared, and then 4 mL of this solution was mixed with methanol extract (40 μL) of the sample. Finally, the samples were incubated for 30 min in the dark at room temperature. Scavenging capacity was read by spectrophotometer (PerkinElmer Lamda 950 UV/Vis/NIR) by monitoring the decrease in absorbance at 517 nm. This absorption maximum was first verified by scanning freshly prepared DPPH from

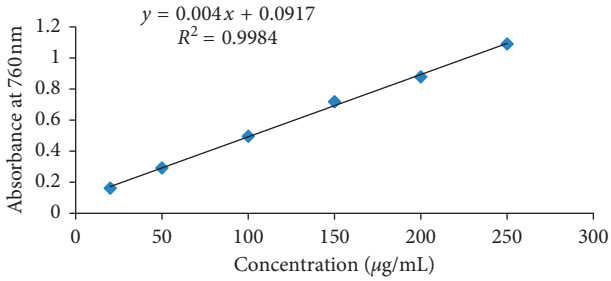

Figure 2: Gallic acid standard curve for the calculation of total polyphenols content.

200–800 nm using the scan mode of the spectrophotometer. Free radical scavenging activity DPPH in percent (%) was then calculated:

$$\text{radical scavenging activity}\,(\%) = \frac{(A_o - A_1)}{A_o} * 100, \quad (2)$$

where A_o is the absorbance of the control and A_1 is the absorbance of the sample.

2.6. Moisture Content. Before and after plasma treatment, whole peanut from treatment (triplicate) was dried in a forced air oven at 130°C for 6 hours [32]. The weight differences before and after oven drying will be used to calculate moisture content (MC; % dry weight).

2.7. Acid and Peroxide Values. Acid value in mgKOH g^{-1} oil and peroxide value in $mEqO_2\,kg^{-1}$ oil were determined according to standard methods (AOAC, 2010).

2.8. Fatty Acid Determination. The lipid fraction of peanut seed oil samples was extracted and fatty acids methyl esters were prepared [33], and the fatty acid profile was determined by gas chromatography with a mass spectrophotometer (GC-MS).

2.9. Statistical Analysis. Data were subjected to the analysis of variance test (one-way ANOVA) using the JMP 7.01 SAS Institute Inc., 2007 software computer package. A comparison test on treatment means was conducted using the post hoc Tukey test at ($p < 0.05$) differences with 95% confidence level.

3. Results and Discussion

3.1. Fatty Acid Profiles. Surface oxidation and development of undesirable changes may occur in food from extreme doses of cold plasma, and cold plasma generates free radicals and reactive species that may modify the functions of fatty acids, inducing lipid oxidation [15]. However, several authors have reported that atmospheric cold plasma treatment did not cause any negative effects on the chemical quality of food products.

Table 1 shows fatty acid compositions of peanut oils variations depending on the cold plasma conditions. The fatty acids identified from untreated (control) peanut oil were 13.34% palmitic acid (C16 : 0), 4.47% stearic acid (18 : 0), 43.46% oleic acid (C18 : 1), 32.56% linoleic acid (18 : 2), 1.35% arachidic acid (20 : 0), 1.39% gadoleic acid (C20 : 1), and 2.89% behenic acid(22 : 0). This is in agreement with previously reported data [1, 34]. The major fatty acids of the unsaturated fatty acids suggest that the peanuts oil is highly nutrient. The ratio of oleic-to-linoleic acid (O/L) is a quality index employed to decide peanut shelf-life and oil stability, ranging from 1 to 1.5, 1.5 to 9.0, and above 9.0, classified as normal, mid, and high-oleic type, respectively [35]. The present study was carried out with normal oleic peanuts (O/L = 1.335). The total saturated fatty acids and unsaturated fatty acids in oil extracted from nonplasma-treated (control) samples of peanut seed oil was 21.92% and 77.41%, respectively.

Palmitic acid contents of all treatments ranged from 13.34% (control) to 15.23% (cold plasma treated).This type of fatty slightly increased in all cold plasma operating conditions but there were no significant ($p > 0.5$) differences between all samples (Table 1). Stearic acid contents of untreated and cold plasma treated peanut samples were found increased but utmost nonsignificant ($p > 0.05$) in all experiments. In addition, while oleic acid contents of untreated peanut oil samples change between 43.47% (control) and 35.74% (plasma treated), linoleic acid contents of peanut oils ranged between 32.56% (control) and 24.49% (plasma treated).

Oleic and linoleic acid content was decreased, and significant ($p < 0.05$) difference was observed at different plasma operating conditions (plasma power, air flow rate, and treatment time). The same result was reported by Albertos et al. [36]; the cause might be reaction produced by the H and OH plasma species. Gadoleic acid (C20 : 1) is one of the unsaturated types of fatty acid and occurs in minor proportions. During this experiment, its amount was decreased but there was no significant ($p > 0.05$) difference throughout the experiments as shown in Table 1. Significant increase in behenic and arachidic acids at various plasma parameters rates in peanut seed oils and significant difference ($p < 0.05$) was observed between same treatments. Generally, a slight increase in saturated fatty acids and a decrease in unsaturated fatty acids were observed during the experiment (Table 1). The results at 25 W, 10 L/min, and 1 min were similar to the control as shown in Table 1. This might be the reaction between the sample, and the energetic particles especially oxygen reacting species from plasma was short, thus leading fatty acid profiles to remain unaffected.

The available studies on the effects of cold plasma on lipids in different food products are very limited. However, based on the reported studies, treatment time and plasma gas could be considered as critical factors affecting lipid oxidation [37]. According to Cämmerer and Kroh [38], conventional roasting at 120–160°C for long time treatment, the structure of lipid storage cells is damaged and oil exposure to oxidation rate increase, but as indicated in Figure 3, in this study, the variation of temperature the ceramic of the cold plasma was below 80°C; therefore, atmospheric cold plasma would significantly decrease the risk of oil

Table 1: Mean values comparison of fatty acid profiles of the cold plasma-treated peanut ($p < 0.05$).

	Plasma treatment condition	C16 : 0	18 : 0	C18 : 1	C18 : 2	20 : 0	20 : 1	22 : 0
1	34 W, 16 L/min, 12 min	14.32 ± 1.84^{a}	7.12 ± 0.35^{a}	38.19 ± 1.63^{c}	28.00 ± 1.41^{bcd}	4.09 ± 0.14^{abc}	1.08 ± 0.25^{a}	5.73 ± 0.66^{ab}
2	34 W, 16 L/min, 4 min	14.22 ± 0.35^{a}	6.14 ± 1.21^{ab}	38.42 ± 0.71^{bc}	29.06 ± 0.14^{abc}	3.99 ± 0.14^{abc}	1.06 ± 0.21^{a}	$5 \pm 0.01a^{bc}$
3	25 W, 10 L/min, 15 min	14.00 ± 0.14^{a}	6.24 ± 0.35^{ab}	38.36 ± 2.05^{bc}	25.76 ± 0.92^{bcd}	5.73 ± 0.85^{a}	0.29 ± 0.28^{a}	5.54 ± 0.64^{ab}
4	25 W, 20 L/min, 8 min	15.08 ± 0.21^{a}	7.01 ± 0.02^{a}	37.12 ± 2.97^{c}	25.04 ± 0.92^{bcd}	4.89 ± 0.28^{abc}	0.54 ± 0.64^{a}	7.53 ± 0.46^{a}
5	34 W, 4 l/min, 4 min	15.23 ± 0.42^{a}	7.03 ± 0.19^{a}	37.40 ± 0.71^{c}	26.62 ± 0.85^{bcd}	4.03 ± 0.07^{abc}	0.97 ± 0.07^{a}	6.93 ± 1.34^{a}
6	10 W, 10 L/min, 8 min	14.45 ± 0.78^{a}	6.07 ± 0.14^{ab}	39.39 ± 0.71^{abc}	29.93 ± 1.32^{ab}	3.56 ± 0.49^{bc}	1.06 ± 0.14^{a}	3.84 ± 1.16^{bc}
7	25 W, 10 L/min, 8 min	14.32 ± 0.46^{a}	6.40 ± 0.70^{ab}	39.38 ± 0.71^{abc}	26.98 ± 0.67^{bcd}	3.42 ± 0.62^{c}	1.05 ± 0.13^{a}	5.66 ± 0.49^{ab}
8	25 W, 0.5 L/min, 8 min	13.99 ± 0.07^{a}	7.10 ± 0.28^{a}	39.7 ± 0.86^{abc}	26.59 ± 0.78^{bcd}	3.43 ± 0.62^{c}	0.98 ± 0.07^{a}	6.53 ± 0.57^{a}
9	16 W, 4 L/min, 4 min	14.13 ± 0.42^{a}	5.42 ± 0.50^{ab}	37.60 ± 0.64^{c}	29.68 ± 0.71^{ab}	4.04 ± 0.14^{abc}	0.96 ± 0.11^{a}	3.48 ± 0.57^{bc}
10	25 W, 10 L/min, 1 min	13.24 ± 0.35^{a}	4.51 ± 0.35^{b}	43.27 ± 0.99^{ab}	32.46 ± 0.85^{a}	1.60 ± 0.26^{d}	1.26 ± 0.28^{a}	2.87 ± 0.19^{c}
11	16 W, 16 L/min, 4 min	14.11 ± 0.21^{a}	6.37 ± 0.42^{ab}	36.89 ± 0.28^{c}	29.41 ± 0.64^{abc}	4.43 ± 0.71^{abc}	1.14 ± 0.21^{a}	5.03 ± 0.06^{abc}
12	16 W, 16 L/min, 12 min	14.29 ± 0.35^{a}	7.19 ± 0.96^{a}	38.88 ± 1.23^{abc}	26.26 ± 1.2^{bcd}	4.07 ± 0.21^{abc}	0.52 ± 0.52^{a}	6.68 ± 0.54^{a}
13	40 W, 10 L/min, 8 min	15.06 ± 0.09^{a}	6.98 ± 0.14^{a}	35.74 ± 1.21^{c}	24.49 ± 2.04^{d}	5.34 ± 0.71^{ab}	0.32 ± 0.85^{a}	7.4 ± 0.69^{a}
14	34 W, 4 L/min, 12 min	14.27 ± 1.03^{a}	7.41 ± 0.49^{a}	36.85 ± 0.47^{c}	25.58 ± 20^{bcd}	5.23 ± 0.42^{ab}	0.11 ± 0.17^{a}	7.45 ± 0.71^{a}
15	16 W, 4 L/min, 12 min	15.00 ± 0.02^{a}	7.50 ± 0.69^{a}	36.27 ± 0.94^{c}	24.99 ± 0.07^{cd}	5.04 ± 0.06^{abc}	0.48 ± 0.57^{a}	6.92 ± 0.49^{a}
16	Control	13.34 ± 0.85^{a}	4.47 ± 0.32^{b}	43.46 ± 0.72^{a}	32.56 ± 1.13^{a}	$1.35 \pm .01^{d}$	1.39 ± 0.49^{a}	2.89 ± 0.27^{c}

All values are mean ± SD. $^{a-d}$Values in the same column with different superscripts are significantly different.

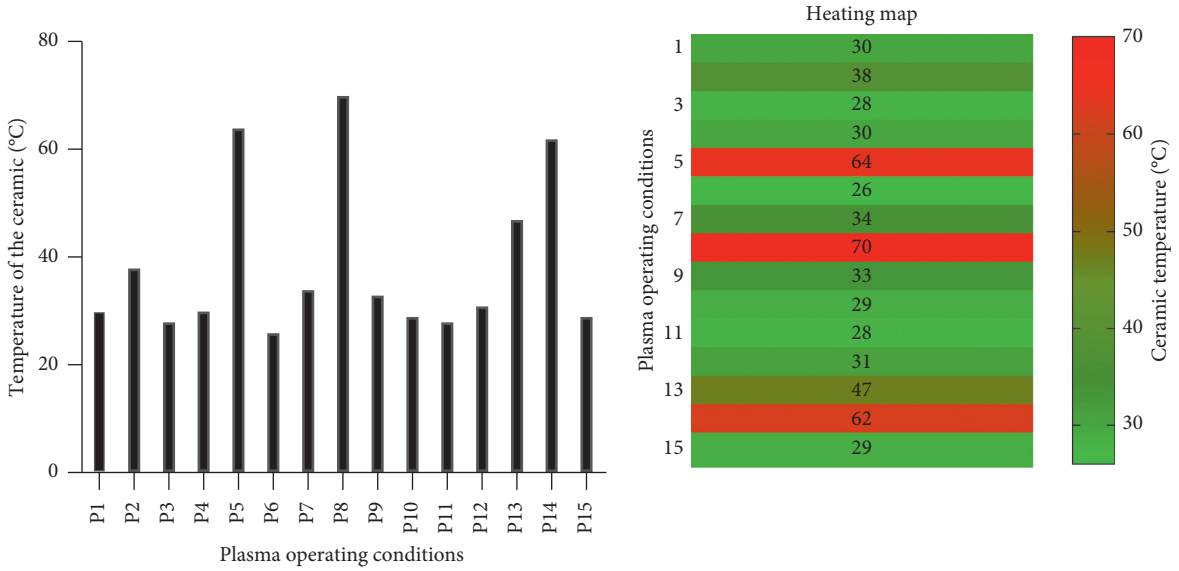

Figure 3: Temperature of ceramic at different plasma operating conditions.

exposure to thermal oxidation. The changes in fatty acid compositions by application of cold plasma could be due to the detrimental effect of the reactive species of cold plasma [21]. Recently, Sarangapani et al. [39] have indicated that cold plasma oxidation of lipids.

Plasma treatment produces free radicals such as hydroperoxyl radicals, superoxide radicals, and singlet oxygen that attack unsaturated fatty acids, which causes to decreases and increased total saturated fatty acids gradually [21]. According to another study by Mexis and Kontominas [40], monounsaturated fatty acids, as opposed to polyunsaturated fatty acids, were preferentially attacked by oxygen to produce primary and secondary oxidation products under gamma irradiation. Irradiation caused a significant gradual decrease in the unsaturated fatty acid content and a significant saturated fatty acid content increase as irradiation dose increased in sesame seeds [41]. Another study suggested that the decrease in unsaturated fatty acids during the irradiation exposure of oil was mainly due to a molecular structure change in fatty acids [42].

3.2. Acidity Value (AV). Acidity value is an indicator for edibility of oil and suitability for industrial use and any extreme change could lead to an unwanted influence on the sensory acceptability and shelf-life of the treated food product. Peanut is a high oil content product (50–55%), with high unsaturated fatty acids, which are susceptible to oxidation [43, 44]. The oil extracted from untreated (control) peanut seeds has an acid value of 0.82 mg KOH g^{-1} (Table 2), which is already in use for edible purpose, and this falls within the recommended by Alimentarius codex [45]. Results obtained from this work indicated that the acid value of the peanut oil corresponds to low levels of free fatty acids

Table 2: Mean values comparison of chemical and antioxidant properties of cold plasma-treated peanut ($p < 0.05$).

	Plasma treatment condition	DPPH (%)	PV (mEq $O_2 kg^{-1}$)	AV (mg $KOHg^{-1}$)	TPC (mg gallic acid/100 g)	MC (%)
1	34 W, 16 L/min, 12 min	93.29 ± 0.35^{c}	2.30 ± 0.11^{e}	3.12 ± 0.18^{a}	213.48 ± 0.71^{ef}	4.67 ± 0.08^{cd}
2	34 W, 16 L/min, 4 min	94.32 ± 0.21^{ab}	2.53 ± 0.09^{e}	1.40 ± 0.15^{bc}	200.73 ± 1.41^{gh}	4.88 ± 0.03^{bc}
3	25 W, 10 L/min, 15 min	94.67 ± 0.28^{a}	2.33 ± 0.05^{e}	1.06 ± 0.11^{c}	200.20 ± 0.64^{gh}	4.89 ± 0.14^{bc}
4	25 W, 20 L/min, 8 min	94.32 ± 0.07^{ab}	2.40 ± 0.09^{e}	1.11 ± 0.16^{c}	197.45 ± 3.77^{h}	5.20 ± 0.03^{ab}
5	34 W, 4 l/min, 4 min	94.41 ± 0.21^{ab}	8.33 ± 0.51^{c}	3.05 ± 0.09^{a}	226.05 ± 3.50^{de}	4.5 ± 0.013^{d}
6	10 W, 10 L/min, 8 min	94.42 ± 0.35^{a}	2.33 ± 0.05^{e}	1.05 ± 0.08^{c}	202.05 ± 3.22^{fgh}	5.17 ± 0.08^{ab}
7	25 W, 10 L/min, 8 min	94.9 ± 0.07^{a}	2.78 ± 0.04^{e}	1.10 ± 0.16^{c}	212.70 ± 4.17^{fg}	5.19 ± 0.08^{ab}
8	25 W, 0.5 L/min, 8 min	93.25 ± 0.07^{c}	13.95 ± 0.86^{a}	3.16 ± 0.12^{a}	341.15 ± 2.12^{a}	3.30 ± 0.01^{f}
9	16 W, 4 L/min, 4 min	94.47 ± 0.42^{ab}	1.71 ± 0.29^{e}	1.12 ± 0.17^{c}	202.24 ± 3.17^{fgh}	5.28 ± 0.02^{a}
10	25 W, 10 L/min, 1 min	94.75 ± 0.14^{a}	1.59 ± 0.13^{e}	0.84 ± 0.08^{c}	202.7 ± 3.61^{fgh}	5.33 ± 0.04^{a}
11	16 W, 16 L/min, 4 min	94.73 ± 0.21^{a}	2.44 ± 0.07^{e}	1.05 ± 0.22^{c}	199.23 ± 1.41^{h}	5.29 ± 0.10^{a}
12	16 W, 16 L/min, 12 min	94.37 ± 0.14^{ab}	2.17 ± 0.38^{e}	1.15 ± 0.05^{c}	194.11 ± 5.47^{h}	4.88 ± 0.15^{bc}
13	40 W, 10 L/min, 8 min	94.69 ± 0.07^{a}	2.37 ± 0.19^{e}	1.53 ± 0.49^{bc}	243.92 ± 5.56^{c}	4.40 ± 0.12^{d}
14	34 W, 4 L/min, 12 min	93.59 ± 0.28^{bc}	6.81 ± 0.69^{d}	3.29 ± 0.68^{a}	303.98 ± 2.83^{b}	3.42 ± 0.11^{f}
15	16 W, 4 L/min, 12 min	94.13 ± 0.07^{abc}	10.20 ± 0.15^{b}	2.41 ± 0.60^{ab}	230.54 ± 2.08^{d}	3.91 ± 0.01^{e}
16	Control	94.72 ± 0.35^{a}	1.56 ± 0.20^{e}	0.82 ± 0.22^{c}	200.23 ± 1.41^{gh}	5.38 ± 0.10^{a}

All values are mean ± SD. $^{a-h}$Values in the same column with different superscripts for each type of analysis are significantly different. DPPH, 1,1-diphenyl-2-picrylhydrazyl; PV, peroxide value; AV, acid value; TPC, total phenolic content; MC, moisture content.

present in the oil in most experiment trials, which suggested low levels of hydrolytic and lipolytic activities in the oil.

The acid value of the oil extracted from noncold plasma-treated peanut oils samples increased from 0.82 ± 0.22 to 3.16 ± 0.12 mg KOH g^{-1} oil during the treatment. The increase in the acid value of oil during the treatment might be due to slight and random hydrolysis of triglycerol molecules to free fatty acids and diacylglycerols [46]. Recently, Kim et al. [47] evaluated the physicochemical characteristics of milk that was treated with cold plasma and reported an increase in acidity. When peanut seeds were treated with optimum cold plasma condition rates, the fatty acid was oxidized rapidly, and the AV would increase. It is clear that no significant difference ($p > 0.05$) was observed between treated and untreated groups (Table 2) except at extreme conditions. The results demonstrate that the peanuts treated under the optimal cold plasma conditions were stable in the acid value.

3.3. Peroxide Value (PV). Lipid oxidation is a complex process involving free radical chain mechanisms forming fatty peroxidation products [48] and peroxide (PV) important parameters for elucidating the peanut oil quality and assessing the oxidation extent [49]. Since cold plasma is often considered as an advanced ionized new technology, it is important to analyze its influence on the lipids present in the fatty foods. As Table 2 indicates, the PV produced from control and cold plasma-treated peanut oil was almost below 10 mEqO_2 kg^{-1} oil except for few experiment trials, and it is low as the Codex Alimentarius Commission stipulated permitted maximum peroxide levels of 10 mEqO_2 kg^{-1} oil [45]. As the plasma power and treatment time increased, the air flow rate decreased, the overall lipid oxidation increased, and significantly different ($p < 0.05$) from other plasma operating conditions.

Different researchers have done different experiments and have reported different results. After cold plasma treatment in fresh and frozen pork [50], beef jerky [25], and raw pork [51] have observed no significant effect on lipid oxidation. However, in [52] an increase has been reported in lipid oxidation in fresh pork and beef after treating them for an extended time period. Recently, Albertos et al. [36] have reported that cold plasma treatment led to a significant lipid oxidation in fresh mackerel fillets. It has been reported in [47, 52] that plasma treatment of meat products increased lipid oxidation when subjected to higher treatments.

A comparison of different voltages and treatment time showed both variables increased the rate of oxidation [36]. Joshi et al. [53] also suggested that lipid oxidation is proportional to the amount of plasma energy applied. Van Durme et al. [54] also revealed that cold plasma caused the formation of many volatiles related to lipid oxidation. During this study, the peroxide value of the oils tested significantly increased ($p < 0.05$) (an increase from 1.56 to 13.95 mEqO_2 kg^{-1} oil), which might be attributed the lack of optimum operating conditions of cold plasma. Cold plasma can generate reactive (free radicle) species that have strong oxidation capacities and that cause lipid oxidation [24]. Thirumdas et al. [55] reported that the main problem encountered was an increase in PV which is at higher power and treatment time. Similar results were observed in the case of our results cold plasma-treated peanuts samples.

3.4. Total Polyphenols. Polyphenols are common constituents in plant products and important antioxidants, which are contained, in large amounts, in peanut [56] and used as antifungal infections in peanuts. Polyphenols play a role in the prevention of degenerative diseases, mainly cardiovascular diseases and cancers with their antioxidative properties [57].

In this study, polyphenols were used as indicators to assess the degree of oxidation by cold plasma. Total polyphenol of untreated and cold-plasma treated peanut seeds is shown in Table 2. The total polyphenol content of untreated (control) peanut seeds was 200.23 mg Gallic acid 100^{-1}. This amount is similar to that in the literature [58–60]. In this study, there was a variation in total polyphenol contents and significant variations between untreated and cold plasma

treated ($p < 0.05$). The reported results on the effects of cold plasma treatment on the total phenolic contents of the food products have a wide degree of variation. A decrease in the total polyphenols was reported in orange juice [61], white grape juice [12], and lamb's lettuce [62]. On the other hand, no significant effect in apples [63] but a significant increase in cashew apple juice [64] and blueberries [65] were also reported. Recent studies using microwave plasma treatment of mandarins increased the total phenolic content [66].

Garofulić et al. [9] studied the effect of atmospheric-pressure plasma treatment on the phenolic acids of sour cherry Marasca juice, the result reveal that enhanced the concentration of phenolic acids. Herceg et al. [67] evaluated the effect of gas plasma on the phenolic content of pomegranate juice, and an increase in total phenolic content was observed. As Table 2 shows, in some experiments, phenolic content was increased. UV radiations and reaction oxygen species formed may be responsible for the increasing phenolic compounds which are extracted from the upper cells because phenols protect cells against the damaging effects of external stress such as reactive oxygen species.

Therefore, the amounts of polyphenols may vary depending on the cold plasma operating conditions applied, and total polyphenols were not affected by cold plasma under the optimal conditions. Most setups as shown in Table 2 except 34 W, 16 L/min; 12 min; 34 W, 4 L/min, 4 min; 25 W, 0.5 L/min, 8 min; 34 W, 10 L/min, 8 min; 40 W, 4 L/min, 12 min, and 16 W, 4 L/min, 12 min were optimum when compared to the control.

3.5. Antioxidant Activity. Although antioxidant activity is not a direct quality attribute used in the food industries, it is a close indicator of various polyphenols present in the food products. The antioxidant effects of phenolic compounds could be due to their redox properties, which include possible mechanisms such as free-radical scavenging activity, transition metal-chelating activity and singlet-oxygen quenching capacity [68].

There was no significant difference ($p > 0.05$) in antioxidant activity between utmost cold plasma operating conditions as indicated in Table 2 during this research study. In previous research, no significant changes in the antioxidant capacity after cold treatment were reported in radish sprouts, kiwifruits, red chicory, and onion powder [69–72]. However, some studies have shown a reduction in antioxidant activity after cold treatments in apples, white grape juice, and cashew apple juice on an extended exposure [12, 63, 64]. Almeida et al. [61] reported a reduction in the antioxidant capacity of prebiotic orange juice after a direct mode of plasma treatment, whereas insignificant effects were reported when treated under indirect mode.

3.6. Moisture Content. Attree et al. [58] reported the moisture content of raw peanut seed ranged from 5 to 6%, and our result was 5.38% as indicated in Table 2. The moisture loss was found to be a function of the linear effect of power, air flow rate, and treatment time and a significant ($p < 0.05$) difference was observed (Table 2). The causes of loss in the moisture of the peanut are the interaction of ions, electrons, and energetic species of neutral atoms, and UV-Vis radiations cause a rapid removal of low molecular contaminants such as additives, processing aids, and adsorbed species. The moisture content of peanut is a critical factor to be measured and controlled in its marketing, processing, and storage [73]. Additionally, it has a profound effect on its characteristics, texture, palatability, consumer preferably, and preservation time, and related studies indicated that moisture content accelerated the process of oxidative rancidity reactions and further affected the product taste when the moisture is too high or too low, but during this study, the moisture of the peanut was not severely reduced and it is near to the optimum moisture content of peanut for storage (5.15%) according to [74].

According to Thirumdas et al. [18], plasma treatment loss of moisture from the surface was due to etching. Therefore, it was observed that the moisture loss increases with an increase in plasma power, treatment time, and decreases in air plasma rate. Moisture loss depends mainly on water loss, and it is important because it affects the visual appearance and texture of the peanut and causes a reduction in saleable weight.

4. Conclusion

The applications of plasma in the food industry is still an emerging field with promising results for fast, effective, safe, and green modification of food. It was shown that the PV, AV, total polyphenols, antioxidant activity, moisture content, and fatty acid values were analyzed using cold plasma, where slight changes were observed on some physical parameters. The most important finding of this research was the observation of the strong relationship between power plasma, air flow rate, and treatment time toward the effect on peanut quality. From this study, it is possible to build a better understanding of how the quality parameters of peanuts are subjected to atmospheric plasma treatment conditions and could help to obtain the optimum condition of plasma power, air flow rate, and treatment time.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

In-Package Air Cold Plasma Treatment of Chicken Breast Meat: Treatment Time Effect

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The objective of this study was to investigate the effects of in-package dielectric barrier discharge (DBD) atmospheric cold plasma (CP) on meat color, microbiological quality and safety of chicken breast meat (pectoralis major). Raw broiler breast meat was collected from a local commercial plant. Noninoculated meat samples and meat samples inoculated with *Campylobacter* and *Salmonella* were packed in polymeric trays with air. The packaged samples were CP-treated at 70 kV for different times (0, 60, 180, or 300 sec) and stored at 4°C for 5 days. Microbial counts (psychrophiles, *Campylobacter, Salmonella*) and meat color (International Commission on Illumination (CIE) $L^*a^*b^*$) were measured before CP treatments and after 5 days of posttreatment storage. Psychrophile growth was inhibited ($P < 0.05$), and both food-borne pathogens were reduced ($P < 0.05$) by more than 90% with CP treatments regardless of treatment time. No differences in pathogenic bacterial counts were observed between the three treatment times; however, increasing treatment time beyond 60 sec resulted in additional inhibition of psychrophilic growth. There were no differences ($P > 0.05$) in a^* and b^* values between pretreatment and posttreatment plus storage; however, all CP treatments resulted in increased L^* value ($P < 0.05$). Results indicate that in-package CP treatments can be used to reduce both microbial spoilage and food-borne pathogen risks, which could increase microbial food safety, although it may result in an overall paler breast meat, and the reduction (about 1 log) in pathogenic and spoilage microbes are limited.

1. Introduction

Microbiological quality and safety of raw poultry meat has been a challenge for the poultry industry. Nonthermal antimicrobial treatments, such as chilling with chlorinated water during processing, and strategies, such as cold-chain management and modified atmosphere packaging (MAP) postpackaging, are used to control microbial quality and safety of fresh poultry meat products during storage. However, they appear either not to be effective against microbial contamination postprocessing or to have limited effects on microbial control during postpackaging handling (marketing and distribution; for example, microbiological shelf life of fresh raw chicken meat is limited to 6–8 day at 4.4°C [1]). Each year, millions of pounds of fresh poultry meat products are lost as a result of microbiological spoilage [2]. In 2011, a potential *Salmonella* contamination resulted in a recall of 36 million pounds of ground raw turkey [3].

In-package dielectric barrier discharge (DBD) atmospheric cold plasma (CP) is a new nonthermal antimicrobial technique for inactivating food-borne pathogens and extending shelf life of fresh food products [4–8]. The CP system consists of two conductive electrodes separated with dielectric layers and a gas gap [4, 8–11]. At high voltage (more than 10,000 kV), this device generates a number of bioactive particles in packages, including reactive oxygen or nitrogen species (RONS, such as ozone, superoxide, hydroxyl radicals, atomic oxygen, nitric oxide, peroxynitrite), ultraviolet (UV), radiation energetic ions, and charged particles [12, 13]. Those particles confer bactericidal, fungicidal, and viricidal effects to the system [14]. For example, UV can cause DNA modification. Charged particles can

cause membrane rupture through electrostatic forces [15]. Guarnieri et al. [16] reported that RONS play a principal role in inactivation of microbes as compared to charged particles and UV in a DBD system. RONS interact with membranes and macromolecules (lipids, proteins, and DNA) and lead to injury and/or death of microbes [17]. The in-package CP allows for the treatment of food products inside sealed packages and eliminates the risk of postprocessing contamination.

A number of studies have documented the efficacy of the in-package DBD CP inactivation of microbes in food products. Misra et al. [6, 18] treated fresh strawberries with the in-package CP and reported that the background microflora (aerobic mesophilic bacteria, yeast, and mold) of strawberries was reduced by 2-log within 24 h of post-treatment, and the effect on product color and firmness was insignificant. Ziuzina et al. [7] treated cherry tomatoes with the same system and found that the treatment for 10, 60, and 120 s resulted in reduction of *Salmonella*, *E. coli*, and *L. monocytogenes* populations on tomatoes to undetectable levels from initial populations of 3.1, 6.3, and 6.7 $\log_{10}$ CFU/sample, respectively. Misra et al. [5] showed that there were no significant differences among weight loss, pH, and firmness between control and treated cherry tomatoes at the end of storage life. Kronn et al. and Wang et al. [4, 19] found that the CP with MAP resulted in more than 4-log reductions in microbial populations and extended microbial quality of fresh chicken meat products compared with untreated samples packaged in ambient air.

Rothrock et al. [11] demonstrated that the in-package CP treatment resulted in more than 2-$\log_{10}$ reduction in spoilage bacterium *Pseudomonas fluorescens* and pathogenic bacterium *Salmonella typhimurium* and more than 4-log reduction in pathogenic bacterium *Campylobacter jejuni* in liquid culture after samples were packed in air and treated at 80 kV for 180 sec. Changes in the CP treatment time significantly influenced CP killing efficacy. In this study, the effects of the CP treatments for different times were further evaluated on food-borne pathogenic and psychrophilic bacteria of packaged raw chicken breast meat (pectoralis major). The effect of the treatments on meat appearance was also estimated using surface color measurements.

2. Materials and Methods

2.1. Meat Samples, Packaging, and Storage. Boneless skinless breast meat (pectoralis major) from broiler birds (35–42 days old) was collected from a local commercial processing facility in Athens, Georgia. A total of 30 cutlets and 20 whole fillets were collected for each trial. Breast cutlets and fillets were transported on ice back to the laboratory and trimmed to remove fat and connective tissue. Prior to treatments, samples were placed in polymeric trays (19.5 cm × 14.5 cm × 4.0 cm, Sealed Air Corp., Duncan, SC, USA). Each tray contained two trimmed fillet cutlets (approximately 90.6 ± 6.5 g each) and two muscle samples (2.5 cm diameter, 2.5–3.0 cm thick, average weight 21 ± 1.5 g) cored from the cranial end of a single whole fillet (approximately 180.1 ± 30.2 g). One cutlet was placed in the tray skin/ventral side up and used for microbial analysis (psychrophiles). The other cutlet was placed in the tray bone/dorsal side up and used for surface color (International Commission on Illumination (CIE) L^*a^* b^*) measurements. One of the muscle core samples was inoculated with *Campylobacter* and the other with *Salmonella* prior to being placed on the tray with the inoculated side up. Individual trays were sealed with a polypropylene-based barrier film (Toplex HB60, Plastopil Europe, Netherlands) in ambient air with a tray sealer (Koch Kats 100 Single Head Tray Sealer, Ultra Source LLC, Kansas City, Missouri, USA). After sealing, samples were let to sit for at least 45 min at ambient temperature so that the relative humidity in the package could reach >80% before CP treatments. After the CP treatments, packaged samples were stored in a 4°C cold room for 5 days before they were evaluated. In each trial, three trays were signed to each CP treatment. A total of three independent trials (with different batches of raw chicken breast meat and on separate dates) were conducted for the whole experiment.

2.2. Inoculation with Food-Borne Pathogens Salmonella and Campylobacter. One *Campylobacter jejuni* isolate and one *Salmonella typhimurium* isolate (both originally recovered from commercial poultry processing environments) were used during this study. The *C. jejuni* isolate was grown in Tryptic Soy Broth/Agar biphasic cultures incubated at 42°C, under a hydrogen-enriched microaerobic atmosphere (7.5% H_2, 2.5% O_2, 10% CO_2, and 80% N_2) for 24 h [20, 21]. The *Salmonella typhimurium* isolate was grown in Tryptic Soy Broth (TSB) at 37°C for 24 h at 200 rpm [22]. After incubation, 5 mL of the *C. jejuni* or *Salmonella typhimurium* growth was added to 495 mL of 1X phosphate buffered saline (PBS) to create the 10^6 CFU/mL inoculum (average 6.6 ± 0.3 log10 for *Campylobacter* and 6.9 ± 0.4 $\log_{10}$ for *Salmonella*). Concentrations of *C. jejuni* and *Salmonella typhimurium* inocula were verified spectrophotometrically (OD600). The inoculation process began by pipetting 75 mL of inoculum into a plastic food tray (CS979, Cryovac, Duncan, SC). Fillet core samples were placed skin side down in each tray so that only the surface sat in the liquid. The core samples soaked for 30 min to allow the microorganisms to attach to the surface. After soaking, the core samples were removed and allowed to drip for 5 min before they were placed in the tray package [4].

2.3. In-Package DBD CP Treatment. The same in-package CP device as described by [4] was used in this study (Figure 1). The treatments were performed with a BK-130 AC dielectric test set consisting of a high-voltage transformer, power supply, and control system. Two 15.24 cm diameter spun-aluminum electrodes (Phenix Technologies, Accident, Md.) were connected to the high-voltage transformer. The two electrodes were arranged parallel to one another on the top and bottom of the sample. The electrodes were separated from the sample package by dielectric barriers and insulated from the bench with a yellow low-voltage electrical blanket (Velcro, 36 × 36 in, Class 0 Type 1, Salisbury standard rubber insulating blanket, Lab Safety Supply, Chicago, Ill.). The top

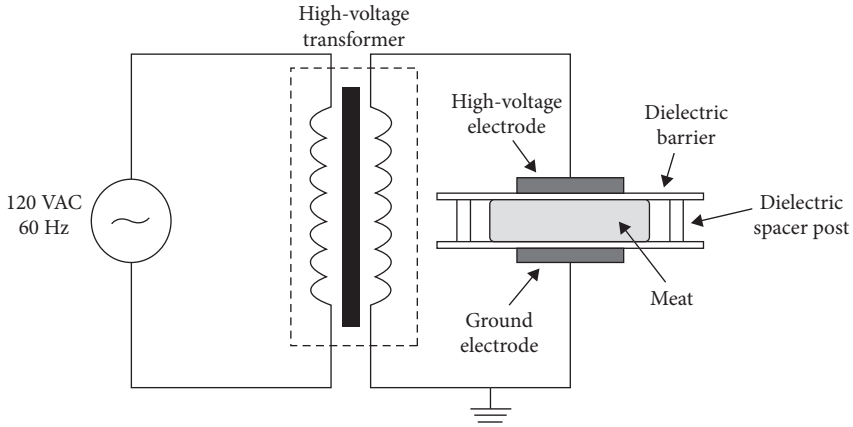

Figure 1: Schematic of in-package cold plasma treatment system.

electrode was connected to the 130 kV tap of the high-voltage transformer with a 1.09 m high-voltage spark plug wire (8.5 mm superconductor spark plug wire, MSD, El Paso, Tex.). For additional insulation, the high-voltage wire was fed through a 1.09 m length of Tygon tubing of 1.27 cm diameter. The bottom electrode was connected to the return terminal of the transformer with the supplied ground wire, and a jumper connected the ground and guard terminals (Figure 1). Before treatments, the system was warmed up with a sealed empty tray at 80 kV for at least 5 min. During the treatments, voltage and current (kV and mA) were monitored, and the beginning and end values were recorded to ensure treatment consistency. Average electric power was 58.5 ± 3.2 W for treatments.

After the meat sample was packaged and sat for more than 45 min, trays were individually treated in the CP device (Figure 1). In this study, only direct treatments (the package and sample are placed directly over the electrode) were tested. Once the tray was placed, the top dielectric barriers (polypropylene sheets) were positioned over the tray, and the electrode and electrode insulator were placed on top of the barriers [4]. Trays were treated for 0, 60, 180, or 300 sec. Temperature and humidity of the laboratory were recorded during every replicate of the experiment [23, 24].

2.4. Ozone Gas Measurement. Ozone gas was used as an indicator for DBD CP generation and antimicrobial activity within the package and commonly measured as a reference for the system performance [4, 7, 8, 19, 25, 26]. Immediately following CP treatment, headspace gas within the sample tray was taken and ozone content in the package was measured with Draeger gas detection tubes (Draeger Safety AG & Co., Lubeck, Germany). To measure high concentrations of ozone in packages, small gas sample volumes (≤3 mL) were collected with a 3 mL syringe. The syringe was flushed with headspace in the package once before gas samples were collected. The needle, which was used to collect ozone gas from package headspace, was removed, and the syringe was connected to a Draeger tube by a 3–4 cm length of flexible tubing. The tube was inserted into a Draeger Accuro detection pump (Draeger Safety AG & Co., Lubeck, Germany). The syringe volume was expelled into the detector tube and then removed, allowing a total flow volume of 100 mL to occur (volume required based on the manufacturer's instructions). The observed gas concentration was then multiplied by the ratio of the detection tube volume over the syringe volume [24]. It is important to note the ozone gas measured by the Drager Ozone Method relies on oxidation of indigo to form isatin [27]. This reaction is not specific to ozone, and many oxidative species will contribute to the "ozone" measurement including superoxide [28], nitrogen oxides [29], and peroxides [30].

2.5. Microbial Analysis. For microbiological recovery, the fillet cutlets or cores (including 3 extra cutlets and 3 meat cores inoculated with *C. jejuni* and 3 meat cores inoculated with *Salmonella typhinurium* for initial microbial load and the samples treated with CP and stored for 5 days) were rinsed with phosphate-buffered saline (PBS, Gibco by Life Technologies, Grand Island, N.Y.) 4 times less than that of meat weight (or about 22.5 mL for a cutlet and 5.25 mL for a cylinder). The PBS diluents were then serially diluted 4 times (~10^7 to 10^3 CFU/mL). *C. jejuni* rinse dilutions were plated onto Campy-Cefex agar, *Salmonella typhimurium* rinse dilutions were plated onto Brilliant Green Sulfa agar (BGS) with 100 ppm nalidixic acid, and psychrophiles were plated onto TSA agar. *C. jejuni* plates and *Salmonella typhimurium* plates were incubated at the same temperature and atmosphere as described in section Inoculation with food-borne pathogens *Salmonella* and *Campylobacter*. For psychrophiles, the plates were incubated at 4°C for 10 days. Plates containing between 30 and 300 colonies were used for enumeration.

2.6. Surface Color Measurement. The surface color ($L^*a^*b^*$) on the bone-side surface of the chicken cutlets was measured using a spectrophotometer (CM-2600d, Konica Minolta, Inc., Tokyo, Japan) with settings of illuminant C, 10° observer, specular component excluded, and an 8 mm aperture. Color was measured before fillets were packed and after the samples were treated and stored at 4°C for 5 days. Surface areas free from obvious defects (bruises, discolorations,

hemorrhages, or any other conditions that might have prevented uniform color readings) were selected for measurements. Two measurements were taken for each fillet cutlet.

2.7. Statistical Analysis. Microbial count data were $\log_{10}$-transformed to normalize the data. Analyses of microbial and quality data were performed using one-way ANOVA via the PROC GLM of SAS (SAS version 9.4, SAS Institute Inc., Cary, NC). Replication was included as a factor. Means were separated using Tukey's multiple comparison tests at a significance level of 0.05.

3. Results

3.1. Ozone Formation in Packages Immediately after CP Treatment. Table 1 shows that ozone concentrations in meat packages treated with the in-package CP were greater ($P < 0.05$) than untreated packages immediately after DBD treatment at 70 kV regardless of treatment time. Increasing CP treatment time from 60 sec to 180 sec further increased ozone concentrations ($P < 0.05$) in packages; however, further increasing the treatment time to 300 s did not significantly increase ozone concentrations.

3.2. Effects of In-Package CP Treatment Time on Microbial Populations. The average populations of psychrophiles on raw fillet cutlets was 4 $\log_{10}$ CFU/mL before packaging and approximately 5 $\log_{10}$ CFU/mL for both *Campylobacter* and *Salmonella* on inoculated meat core samples (Table 2). After 5 days of refrigerated storage without CP treatment, the psychrophilic population significantly increased by ~4 logs (8.33 ± 0.07 CFU/mL; $P < 0.05$); however, *Campylobacter* and *Salmonella* populations were not significantly changed. Bacterial populations on CP-treated chicken breast meat were significantly lower ($P < 0.05$) than that on untreated fillets after 5 days of refrigerated storage, regardless of treatment time or bacterial type (Table 2). Cold plasma treatment for 60 sec resulted in more than 0.5-, 0.7-, and 0.4-log reductions in psychrophiles, *Campylobacter* and *Salmonella*, respectively, compared to nontreated samples ($P < 0.05$). Extending the CP-treatment times beyond 60 sec did not result in any further reductions in the *Campylobacter* or *Salmonella* populations; however, increasing CP-treatment time from 60 sec to 180 sec significantly reduced psychrophilic populations by an additional 0.6 logs. Increasing CP-treatment time did not further reduce psychrophilic populations on the breast meat.

3.3. Effects of In-Package CP Treatment Time on Meat Color. Of the three color measurements, only b^* was significantly affected by the storage time (as noted by the ~2 unit increase in the nontreated control posttreatment sample) (Table 3). When comparing prepackaging to posttreatment readings (Table 3), there were no significant changes in the redness (a^*) or yellowness (b^*) of the CP-treated chicken cutlets for any treatment time, but significant increases in lightness (L^*) (by 3.1–3.6 units) were found for the 60, 180, and 300 sec treatment times. When comparing the effect of treatment time on the posttreatment measurements compared to the no-treatment control (0 sec + 5 d storage), increasing treatment time to at least 180 sec significantly increased L^*, while treatment times of 60 and 300 secs significantly reduced b^*. Redness estimates were unaffected by CP-treatment (Table 3).

Table 1: Effect of cold plasma treatment time on ozone formation (Draeger tube method) in chicken breast fillet packages (mean ± SE).

Treatment (70 kV)	Ozone formation (ppm)
0 sec	0 ± 0^{c}
60 sec	1850 ± 240^{b}
180 sec	2650 ± 145^{a}
300 sec	2550 ± 189^{a}

[a-c]Means within a column lacking a common superscript letter differ significantly ($P < 0.05$).

4. Discussion

The in-package CP device evaluated in this study is based on DBD technology. Dielectric barrier discharges are common methods used to generate plasma at atmospheric pressure [31] and are self-sustaining electrical discharges in electrode configurations containing an insulating material in the discharge path [32]. In DBD, the high electric field or voltage in the discharge gap causes formation of a large number of microdischarges and breakdown of gas molecules through electron-driven ionization and dissociation, creating reactive radicals. The dielectric between the electrode and discharge limits the amount of charge transported by a single microdischarge and distributes the microdischarges over the entire electrode surface area. In literature [5–9, 18, 31, 33], CP formation in packages treated with the same CP system was investigated using optical emission spectroscopy and oscilloscope. Excited species of N_2, NO, N_2^+, O_2^+, and OH group were detected in packages during DBD treatments, and it was concluded that the in-package CP system generated reactive oxygen species and excited nitrogen species. It was also demonstrated that the DBD performance or the DBD-induced CP formation was not affected by the presence of food materials in the packages.

Klockow and Keener [25] concluded that plasma generated from air by an in-package CP device similar to the one used in this study was characterized by generation of various chemically reactive species, with ozone being the most predominant, longest-living, and most oxidative species. Therefore, ozone formation in packages after the in-package CP treatment has been commonly used as an indicator for both CP formation and antibacterial activity [4, 7, 8, 18, 19, 25, 26]. Our study showed that the in-package CP device under the described experimental conditions is very effective in the generation of CP, resulting in antimicrobial activity in packages. Increased treatment times beyond 60 s could further increase the CP formation and enhance antimicrobial activity (especially psychrophiles).

Table 2: Effect of cold plasma treatment time on populations ($\log_{10}$ CFU/mL) of psychrophiles and pathogens on raw chicken breast fillets (mean ± SE).

Treatment (70 kV)	Psychrophiles ($\log_{10}$ CFU/mL)	*Campylobacter jejuni* ($\log_{10}$ CFU/mL)	*Salmonella typhimurium* ($\log_{10}$ CFU/mL)
0 sec + 0 time	4.03 ± 0.34^{d}	5.14 ± 0.14^{a}	5.30 ± 0.31^{a}
0 sec + 5d storage	8.33 ± 0.07^{a}	4.99 ± 0.17^{a}	4.90 ± 0.16^{a}
60 sec + 5d storage	7.81 ± 0.03^{b}	4.20 ± 0.18^{b}	4.49 ± 0.19^{b}
180 sec + 5d storage	7.18 ± 0.12^{c}	3.97 ± 0.13^{b}	4.42 ± 0.21^{b}
300 sec + 5d storage	7.33 ± 0.15^{c}	4.03 ± 0.16^{b}	4.25 ± 0.20^{b}

[a-d]Means within a bacterial populations with no common superscript letter differ significantly ($P < 0.05$).

Table 3: Effect of cold plasma treatment time on CIE L*a*b* values of raw chicken breast fillets (mean ± SE).

	L*		a*		b*	
Treatment (70 kV)	Prepackaging	Posttreatment + 5d storage	Prepackaging	Posttreatment + 5d storage	Prepackaging	Posttreatment + 5d storage
0 sec	56.9 ± 1.0^{bcd}	56.2 ± 0.8^{cd}	-0.31 ± 0.49^{abc}	0.21 ± 0.20^{a}	9.3 ± 0.5^{b}	11.0 ± 0.3^{a}
60 sec	54.2 ± 0.85^{d}	57.8 ± 0.7^{bc}	-0.42 ± 0.2^{abc}	-0.05 ± 0.13^{ab}	8.7 ± 0.4^{b}	9.3 ± 0.4^{b}
180 sec	59.2 ± 0.6^{bc}	62.5 ± 0.7^{a}	-0.80 ± 0.13^{bc}	-0.63 ± 0.14^{abc}	9.5 ± 0.3^{ab}	9.8 ± 0.5^{ab}
300 sec	56.6 ± 1.0^{cd}	59.7 ± 0.7^{ab}	-0.98 ± 0.22^{c}	-0.58 ± 0.18^{abc}	8.4 ± 0.6^{b}	8.51 ± 0.5^{b}

[a-d]Means with no common superscript letter within the same parameter differ significantly ($P < 0.05$). Note: the same chicken breast meat in each treatment was used for color measurements before packaging and after treatment and 5d storage.

However, increases in CP treatment time beyond 180 s at 70 kV did not result in further increases in ozone concentrations in the packages. Similar results have been found in different published studies with the same CP device [8, 19, 26]. The lack of a linear relationship between ozone formation in packages and CP treatment time could be ascribed to quenching by increased levels of water dissociation and to direct reaction of ozone with water, N_2, and/or other active components formed in meat packages with high relative humidity and longer treatment time [10, 31].

Our data demonstrate that an in-package CP system can significantly inhibit growth of spoilage microbes and reduce food-borne pathogens on raw chicken meat surfaces when packaged in air. This result is well in line with the findings published in the literature on antimicrobial effects of CP treatments. Kim et al. [34] found that total bacterial populations on sliced bacon packed in helium and the helium/oxygen mixture decreased by 1.89 and 4.58 log CFU per gram, respectively, after CP treatments. Rød et al. [35] reported reductions in *Listeria innocua* populations ranging from 0.8 to 1.6 log CFU per gram after 1 and 14 days of storage, respectively, in the CP-treated, ready-to-eat meat product. Noriega et al. [36] showed that an 8 min CP treatment gave 1-log reduction on chicken skin, and a 4-min treatment gave > 3-log reductions on chicken muscle. Jayasena et al. [13] found that, following a 10 min CP treatment, microbial load reductions in *Listeria monocytogenes*, *Escherichia coli* O157: H7, and *Salmonella typhimurium* were 2.04, 2.54, and 2.68 log CFU per gram in pork-butt samples and 1.90, 2.57, and 2.58 log CFU per gram in beef loin samples, respectively. With the same device, Kronn et al. and Wang et al. [4, 19] also showed that in-package CP treatments significantly reduce microbial growth of total aerobic populations on fresh chicken meat packed under MAP atmospheric conditions. In addition, data from the current study also showed that increasing CP treatment times from 60 to 300 sec does not impact antimicrobial effectiveness against food-borne pathogens; however, increasing treatment time to 180 sec significantly increases antimicrobial efficiency against psychrophiles on raw chicken meat. These results suggest that the antimicrobial effect of the CP treatment time may vary with bacterial type.

Although ozone has been hypothesized to be the main factor contributing to microbial inactivation with DBD CP treatments due to its known antimicrobial properties and its high content within packages [4, 7, 8, 24, 25, 37], data in this study suggest that the antimicrobial role ozone plays in in-package CP treatments may varies with bacteria type. Ozone may have more of an effect on spoilage microbes compared with food-borne pathogens. Vaze et al. [38] concluded that ozone alone might not be a major inactivating factors in CP treatments. With the same device, Rothrock et al. [11] did not observe consistent correlations between bacterial inactivation and ozone contents measured with the Draeger tube method. In fact, plasma chemistry has shown that gas discharges during CP generation can be a source of charged particles, ions, reactive gas species, radicals, and radiation (ultraviolet, infrared, and visible), many of which have biocidal properties [39, 40].

In-package CP treatments in this study consistently and significantly increased the lightness (L^* values) of raw chicken meat but had no effect on redness (a^*) or and yellowness (b^*), regardless of treatment time used in the study. These results indicate that in-package CP treatment may cause changes in the appearances of skinless chicken meat and make raw meat look paler. The effects of CP treatments on meat color have been reported in the past and results showed that the effects varied by treatment/package conditions, meat types, and color parameters. Kim et al. [34] found that L^* values of CP-treated bacon surfaces decreased at a higher input power and greater exposure time; a^* values increased at a higher input power and greater exposure time

in helium gas packages; and b* values did not change when helium was used but increased at a higher input power under helium/oxygen mix. Moon et al. [41] did not observe any large differences in L*, a*, and b* values between CP-treated pork samples and the untreated controls. Increased L* value in CP-treated meat could be attributed to high gaseous ozone formation in packages after CP treatment. Published data have shown that gaseous ozone exposure increased L* value on the surface of chicken breast meat [42] and beef [43].

In conclusion, our data demonstrate that an in-package DBD CP treatment can significantly reduce both spoilage (psychrophiles) and food-borne pathogen (*Campylobacter* and *Salmonella*) populations by as much as 1 log on breast meat packaged in air. The antimicrobial effectiveness against food-borne pathogens is not influenced by cold plasma treatment time (from 60 sec to 300 sec) at 70 kV. However, for spoilage microbes, treatment time may affect the effectiveness of the antimicrobial packaging system. The CP treatment may affect the appearance of raw meat by making the surface paler in color. Further research is needed to minimize the effect of in-package CP treatments on meat color and further enhance its antimicrobial efficiency before it can be applied to extend shelf life of fresh poultry breast meat.

Data Availability

The data used to support the findings of this study are included within the article.

Additional Points

Practical Applications. In-package CP treatments at 70 kV significantly reduces both spoilage (psychrophiles) and food-borne pathogen (*Campylobacter* and *Salmonella*) populations by as high as 90% on raw meat surfaces. For food-borne pathogens, in-package CP treatment time for 60 s is as effective as for 300 s; however, for spoilage microbes, longer treatment times could be more effective. In-package CP treatment may result in significant changes to raw meat appearances.

Disclosure

The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the USDA or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Quality Evaluation of Rice Treated by High Hydrostatic Pressure and Atmospheric Pressure Plasma

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This study applied high hydrostatic pressure (HHP) and atmospheric pressure plasma (APP) treatments to rice and examined the effects of the treatments on the microbial contamination and physicochemical properties. The microbial population was 100% sterilized by HHP and reduced by up to 34% by APP. Color *a* values were increased by up to 285% and 33% in HHP and APP, respectively. HHP increased fructose (~8,256%) but decreased glucose, sucrose, and maltose (~97%, −100%, and −93%, respectively). APP only mildly modified sugar composition compared with HHP. Retrogradation factors were not changed remarkably by HHP or APP. In conclusion, HHP sterilized microorganisms, but the sterilization was accompanied by high modifications to color and sugar composition. APP had a lesser effect on the microbial population, but it only mildly changed the physicochemical properties of the rice. Therefore, application of either HHP or APP could be considered depending on the intended use of the rice.

1. Introduction

Rice (*Oryza sativa* L.) is a major staple crop cultivated in many countries including those of East Asia. Rice cultivation has increased steadily and rice production reached 753 million tons in 2016, of which 686 million tons was harvested in Asian countries [1]. In the food industry, rice utilization has been increasing due to its use as an ingredient in beverages, processed meats, puddings, salad dressings, and gluten-free breads [2]. However, physicochemical modifications are often accompanied by microbial contamination during storage and food processing, which affects the properties of the rice products [3]. Therefore, the importance of quality maintenance has increased with the growth of the rice industry.

Microbial contamination is the major risk factor for crop damage after harvest, thus various strategies have been proposed to prevent such damage [4]. Thermal treatment sterilization is a general method for microbial control, but it induces food color changes, protein denaturation, starch gelatinization, and loss of micronutrients [5]. Therefore, nonthermal treatments such as high hydrostatic pressure (HHP) and atmospheric pressure plasma (APP) treatments have been proposed as alternative strategies. HHP treatment improves storage stability and safety by minimizing damage to micromolecules, including pigments and vitamins. In contrast, macromolecules such as proteins are dissociated by HHP, thus pasteurizing microorganisms [6]. APP treatment creates partially ionized gas containing reactive oxygen species, reactive nitrogen species, and charged particles, as well as ultraviolet radiation [7, 8]. APP interacts with the cell wall and membrane of microorganisms, damaging nucleic acids and proteins. APP has been adapted to inhibit microbial contamination of fresh agricultural products such as cabbage and tomatoes [9].

In this study, we investigated the microbial population and physicochemical properties of rice from seven cultivars

grown in Korea after HHP and APP treatment. The results of this study should be useful for industrial applications through improved safety of rice for storage and processed foods.

2. Materials and Methods

2.1. Sample Preparation and HHP and APP Treatments. The rice cultivars used were the Dabo, Daebo, Sukwang, Sindongjin (Jeonbuk), Samkwang (Chungnam), Jinsumi (Chungbuk), and Haiami (Kyunggi) cultivars, which were grown during the 2016 growing season. The samples were stored in a refrigerator at 4°C until analysis.

The samples were subjected to HHP using a warm isostatic press pressure treatment system (WIP-L60-50-200, Ilshin Autoclave, Inc., Daejeon, Korea), with the temperature of the pressure chamber maintained at room temperature (20°C). A warm isostatic press is a reactor that applies isostatic pressure using water as the pressure medium without the use of heat or gas. It is composed of a high-pressure vessel, a high-pressure pump, a reservoir tank, a safety device, an alarm system, and a control system. The samples were transferred to a laminated aluminum foil film (Newpack, Seoul, Korea) and heat-sealed using vacuum packaging (chamber-type vacuum package, DP-901, Dew Pack Korea Machinery Co., Seoul, Korea). HHP was carried out immediately after germination to prevent enzyme inactivation. The packaged samples were subjected to a pressure of 300 MPa at 25°C for 30 min.

The plasma apparatus used in this study was used previously by Kim et al. [10]. Optimum conditions such as treatment time and input power of APP were established in a previous and preliminary study (data not shown). Briefly, air dielectric barrier discharge plasma source was constructed using a rectangular (parallelepiped) plastic container (137 × 104 × 53 mm). The actuator was made of copper electrodes, and a polytetrafluoroethylene sheet was attached to the inner walls of the container. A bipolar square-waveform voltage at 15 kHz was applied to one electrode, while the other electrode was grounded. The size of powered and grounded electrodes was 30 mm and 10 mm, respectively. Plasma was generated inside the container with an input power of 250 W. Each sample (15 g) was placed in a Petri dish at the bottom of the container, and the distance between the sample and the plasma generator was 20 mm. The sample was treated with the APP source for 20 min.

2.2. Microbial Analysis. The prepared sample (5 g) was mixed for 2 min in a sterile Stomacher bag containing 45 mL of sterile saline solution (0.85%) using a Stomacher Bag-Mixer 400 (Interscience Co., Saint Nom, France). Total plate count agar was prepared for counting of the total number of aerobic microbes (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 37°C for 48 h, and the colony-forming units (CFUs) per gram were counted at a dilution of 30–300 CFU per plate.

2.3. pH. The pH was measured using a pH meter (Model 750; iSTEC, Seoul, Korea). About 1 g of each sample was added to 10 mL of distilled water and homogenized for 30 s. The pH was then measured. Calibration was performed using standard buffers provided by the manufacturer at pH 4, 7, and 10 at room temperature.

2.4. Sugar Content. The free sugar content was measured according to a modification of the method of Woo et al. [11], using fructose, glucose, maltose, and sucrose as standards for calibration curves. Samples were filtered through a 0.45 μm syringe filter (Millipore) and analyzed by high-performance liquid chromatography (HPLC) (Waters 2695; Waters, New Castle, DE, USA). The analytical column was for carbohydrates (4.6 × 150 mm, Waters), and the mobile phase was water-acetonitrile (25 : 75, v/v) at a flow rate of 1 mL/min. The injection volume was 20 μL, and the detector was an evaporative light scattering detector (Waters 2420). All samples were analyzed in triplicate.

2.5. Color. Each sample was poured into a Petri dish, and its color was evaluated using a color difference meter system (Spectrophotometer CM-3500d; Konica Minolta Sensing, Inc., Osaka, Japan). The Hunter color values, L^* (lightness), a^* (redness), and b^* (yellowness), were determined. The instrument was calibrated with a standard black and white plate before analysis. The Hunter values were monitored by a computerized system using SpectraMagic software (Konica Minolta Sensing, Inc.), and the measurements were performed in triplicate.

2.6. Thermodynamic Properties. The thermal behaviors of the samples were determined using differential scanning calorimetry (DSC) (Model Q1000 calorimeter, TA Instruments, Inc., New Castle, DE, USA). Each sample was weighed directly into a DSC pan and distilled water was added to obtain a flour-to-water ratio of 1 : 2.3 (w : w). The pan was then hermetically sealed and allowed to stand for 1 h prior to thermal analysis. Thermal scanning was undertaken from 4°C to 150°C at a heating rate of 5°C/min. The gelatinization onset (T_o), peak (T_p), and conclusion (T_c) temperatures and the transition enthalpy (ΔH) were determined from the peak area of the DSC endotherm.

2.7. Statistical Analysis. Data were presented as the mean ± SD. The Student's t-test was used to compare means between the control group and treatment group. If $p < 0.05$, the result was considered statistically significant.

3. Results and Discussion

3.1. Microbial Population in Rice after HHP and APP Treatments. We determined the effects of HHP and APP on the microbial population in rice from seven cultivars (Figure 1). In the untreated rice group, the microbial concentration was 4.08–4.11 log CFU/g, but APP decreased the population to 2.68–2.84 log CFU/g. The reduction compared to the nontreated rice was 31–34%. The HHP treatment sterilized the microbial contents in the rice from all cultivars

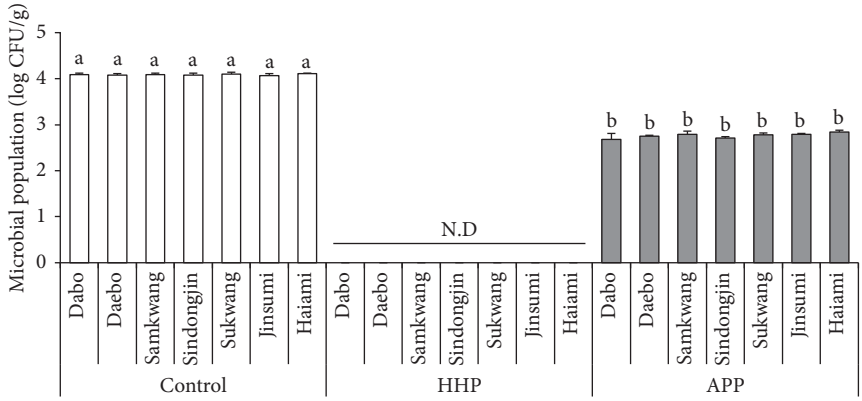


Figure 1: Microbial population (log CFU/g) of rice. Samples were treated by high hydrostatic pressure or atmospheric pressure plasma, and microbial populations were compared to control. Values with different superscripts are significantly different at $p < 0.05$ according to Tukey's multiple range tests within the same cultivar. ND, not detected.

(0 log CFU/g) and was more effective for microbial regulation than APP.

Microbial control is an important issue in food safety. Thermal treatment has been recognized as an effective and economical technique for sterilization; however, it is not suitable for preserving heat-unstable compounds. Therefore, nonthermal treatments for microbial control, including HHP and APP, have received considerable interest [12]. Previously, HHP was used for inactivation of microorganisms and enzymes in legumes and barley and also adapted for modification of allergenic protein in rice [13]. We also confirmed that HHP is effective for microbial inactivation in rice. APP using ionized gas with high kinetic energy has also been implemented for microbe sterilization. In contrast to HHP, APP displays a sterilization effect only against microorganisms on the surface of the material that can come in contact with the plasma gas. Therefore, APP is less effective at sterilizing the inner portion of the material, and this may explain its lower sterilization rate compared to that of HHP [14].

3.2. pH of Rice after HHP and APP Treatments. Microbial growth is affected by environmental factors, including pH [15]. We measured the pH of rice from the seven cultivars after the HHP and APP treatments (Figure 2). The pH in untreated rice was 6.44–6.56, and the pH after APP treatment was 6.39–6.59 which showed only minor changes. The pH of the rice after HHP treatment was 4.55–5.63, a statistically significant ($p < 0.05$) reduction of 13–27% compared with the untreated rice.

HHP treatment induces physical modifications of molecular structures that may alter the chemical compounds that affect pH [16]. However, pH of the rice treated with APP was slightly changed compared to the control, which is interpreted as a result of the low rate of physicochemical changes. Therefore, HHP regulates microorganism population by inducing physicochemical changes, while APP maintains original characteristics of rice and inhibits microbial growth on the surface.

3.3. Sugar Contents of Rice after HHP and APP Treatments. We analyzed the free sugar contents, which determine the quality of rice, by HPLC and expressed the results as the area of the peak (%, Figure 3). The fructose contents were 0.61–6.77% in untreated rice, 87.99–94.00% after HHP, and 0.47–6.94% after APP. The HHP treatment induced a significantly high percentage of fructose compared with both untreated and APP-treated rice. The glucose content was 7.23–19.68% in untreated rice, 0.57–1.51% in HHP-treated rice, and 5.86–19.15% in APP-treated rice. The glucose content was highest in the untreated rice, and the HHP-treated group showed the lowest level compared to the control group and the APP-treated rice ($p < 0.05$). In the sucrose content analysis, the peak areas were 8.73–18.74% for the control group, 0.73–4.28% for the HHP-treated rice, and 7.12–22.60% for the APP-treated rice. The HHP group showed a significantly lower sucrose level ($p < 0.05$) than the control group and the APP group. Lastly, maltose was 46.50–72.27% in the control group, 0.00–0.21% in the HHP group, and 56.46–78.02% in the APP group. The untreated rice contained maltose at a level similar to that of the APP group and higher than that of the HHP-treated rice ($p < 0.05$). To summarize these results, the HHP treatment increased the fructose level compared to the control, but the glucose, sucrose, and maltose levels were decreased significantly. In contrast, the APP treatment showed nonsignificant or minor changes compared with the untreated rice.

HHP preserves the primary structure of molecules and low molecular weight compounds such as vitamins, amino acids, and flavor molecules. However, the secondary and tertiary structures of macromolecules, including starch, can be destroyed [16, 17]. Therefore, the HHP treatment of the rice markedly increased the content of fructose, which has the lowest molecular weight of the sugars. In contrast, the levels of glucose, maltose, and sucrose were decreased by the HHP treatment. Plasma treatment produces reactive species known to react with amylose by depolymerizing, cross-linking, and binding with functional groups and thus modifying the starch structure [18]. However, the APP treatment in our study produced less modification than the

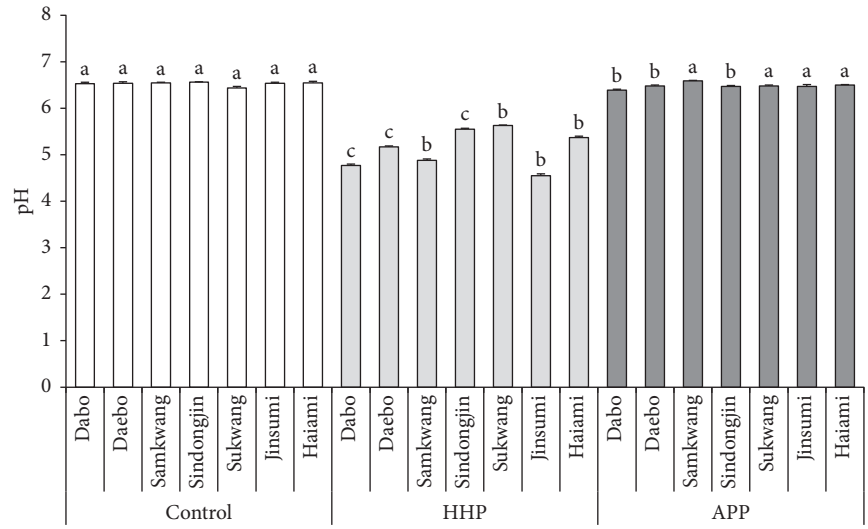
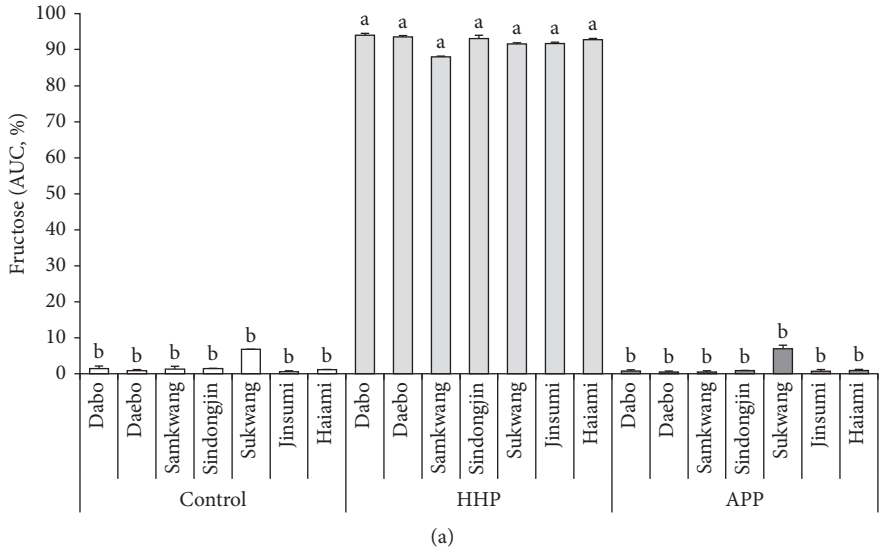
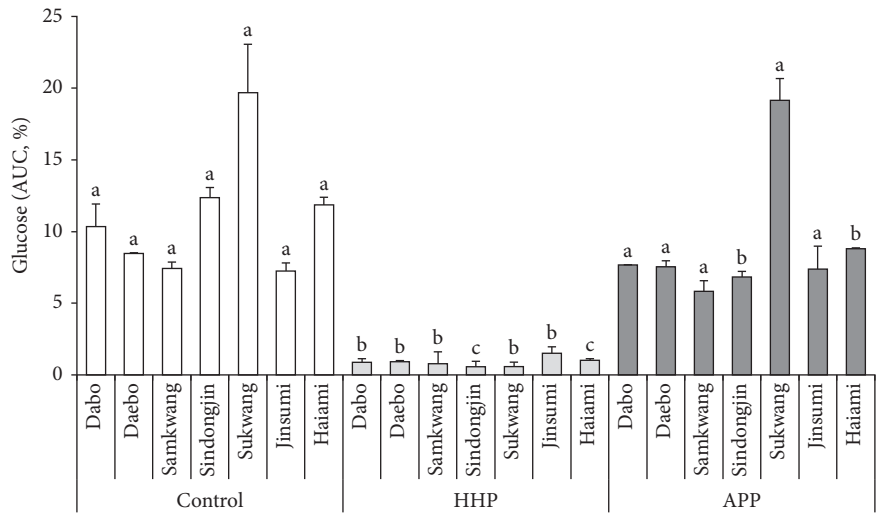


Figure 2: pH changes of rice. Samples were treated by high hydrostatic pressure or atmospheric pressure plasma, and pH changes were compared to control. Values with different superscripts are significantly different at $p < 0.05$ according to Tukey's multiple range tests within the same cultivar.



(a)



(b)

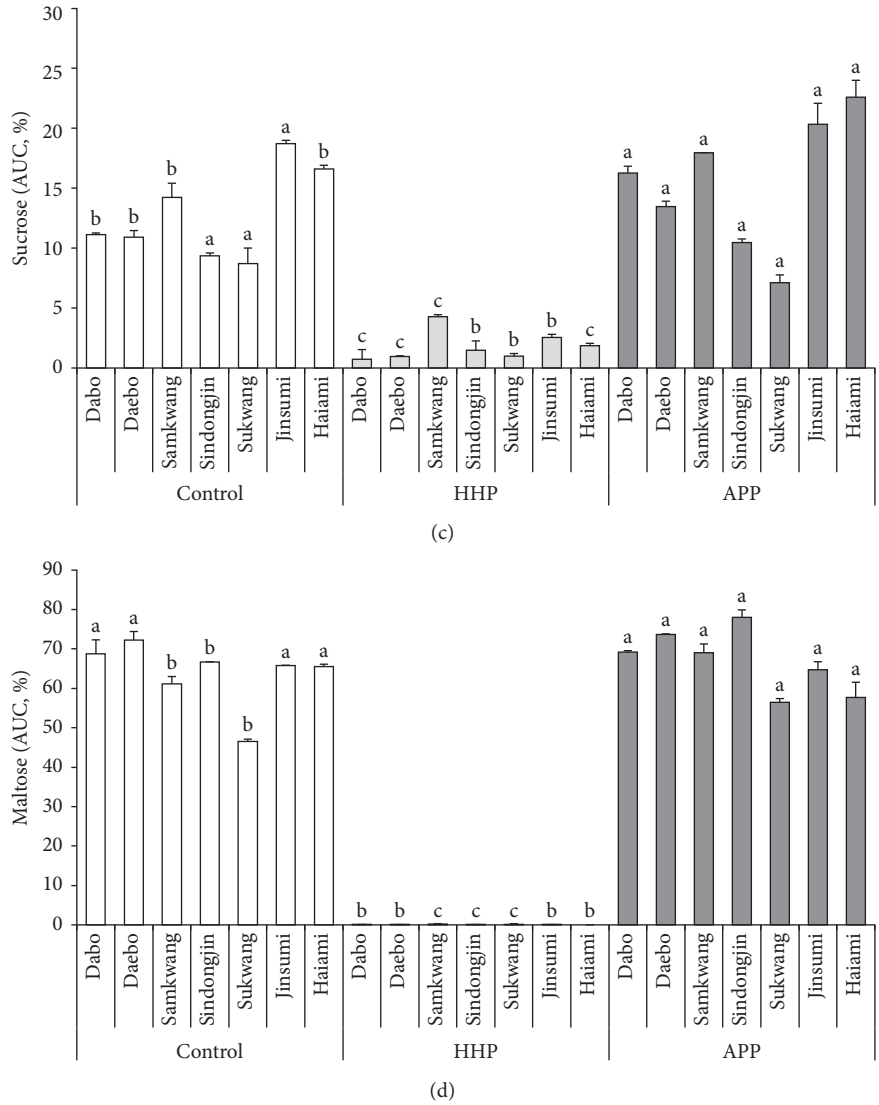


Figure 3: Sugar (fructose, glucose, sucrose, and maltose) contents of rice. Samples were treated by high hydrostatic pressure or atmospheric pressure plasma, and sugar contents were compared to control. Values with different superscripts are significantly different at $p < 0.05$ according to Tukey's multiple range tests within the same cultivar. AUC, area under the curve.

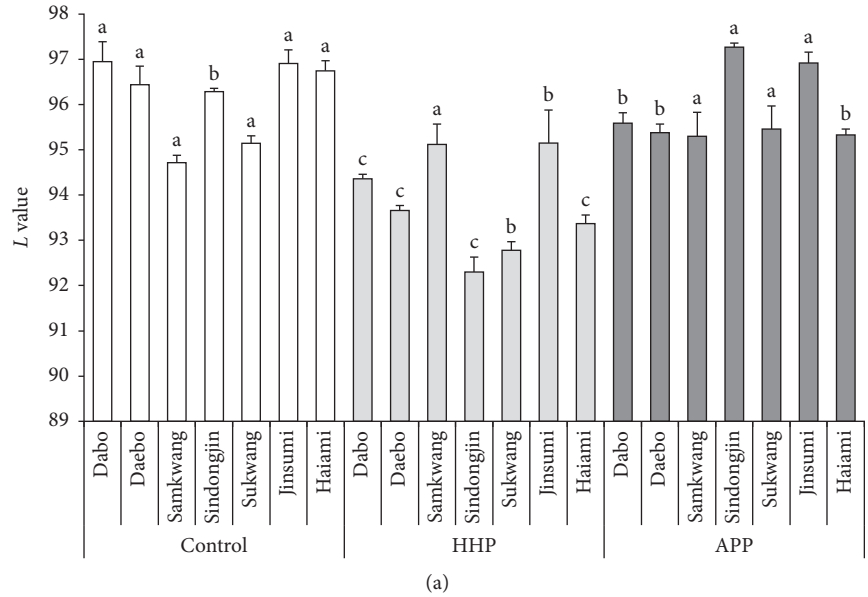


Figure 4: Continued.

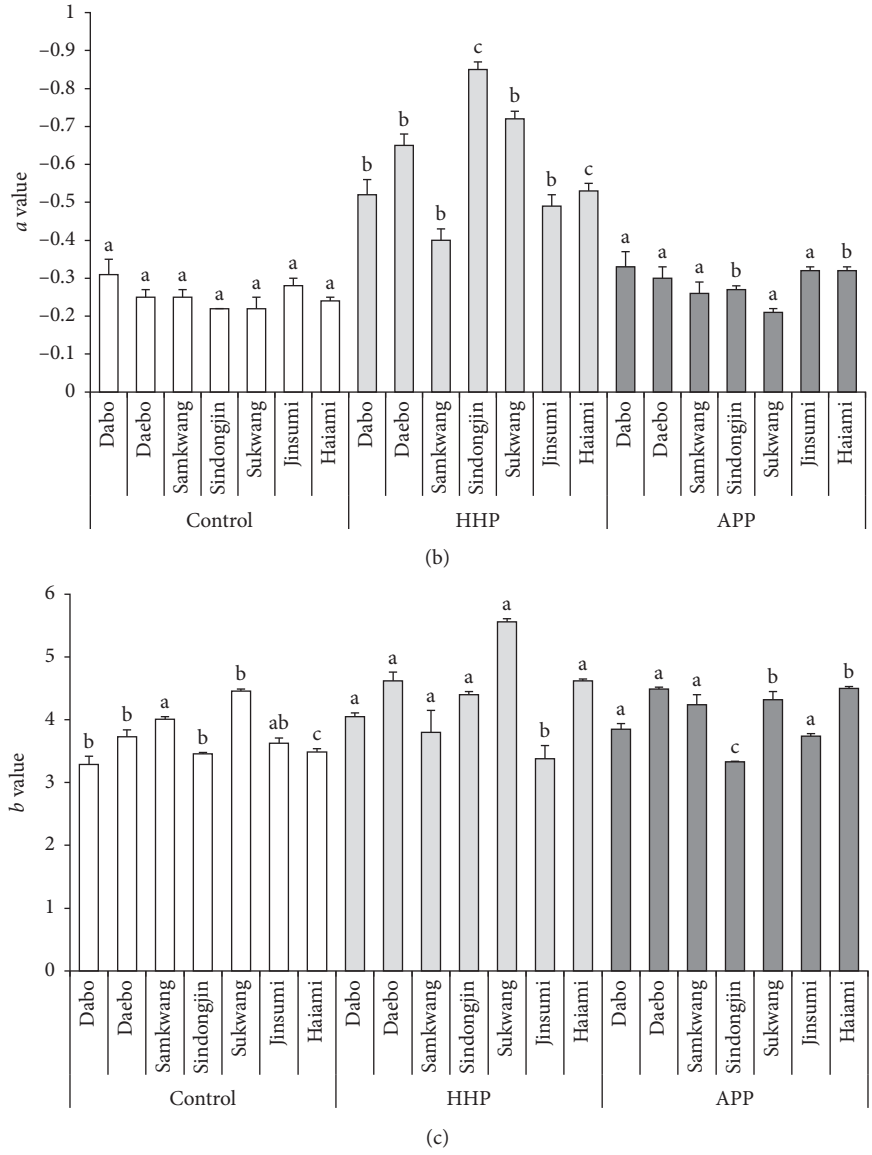


Figure 4: Colorimetry analysis of rice. Samples were treated by high hydrostatic pressure or atmospheric pressure plasma, and colors were compared to control. Values with different superscripts are significantly different at $p < 0.05$ according to Tukey's multiple range tests within the same cultivar.

HHP treatment, which may have been due to an insufficient energy level for interaction with starch molecules inside the material. Therefore, APP modified less sugar composition than HHP, which maintains nutritional value and taste similar to nontreated rice.

3.4. Rice Color after HHP and APP Treatments. We measured Hunter Lab values to confirm the effects of HHP and APP on the color of the rice (Figure 4). The brightness (L) value was 96.95–94.72 in the control, 95.15–92.30 in the HHP group, and 97.27–95.30 in the APP group. The Dabo, Daebo, Sindongjin, and Haiami cultivars showed significant differences compared to the control after HHP and APP, but the Sukwang and Jinsumi cultivars were significantly lower for only the HHP group ($p < 0.05$). The Samkwang cultivar showed no significant change in brightness with either HHP or APP. The a values, which indicate the degree of redness, were −0.22 to −0.31, −0.40 to −0.85, and −0.21 to −0.33 in the control, HHP, and APP groups, respectively. The APP-treated rice showed significantly low values in only the Sindongjin and Haiami cultivars. In the HHP-treated rice, the a value was decreased in every cultivar, indicating induction of greenness. The b value, indicating the degree of yellowness, was also measured. The ranges of the b value were 3.29–4.46, 3.38–5.56, and 3.33–4.50 in the control, HHP, and APP groups, respectively. The HHP treatment significantly increased the b value, except in the Samkwang and Jinsumi cultivars. The APP treatment increased the b value in the Dabo, Daebo, and Haiami cultivars. In conclusion, the HHP treatment produced a decrease in brightness and redness, and an increase in yellowness. The

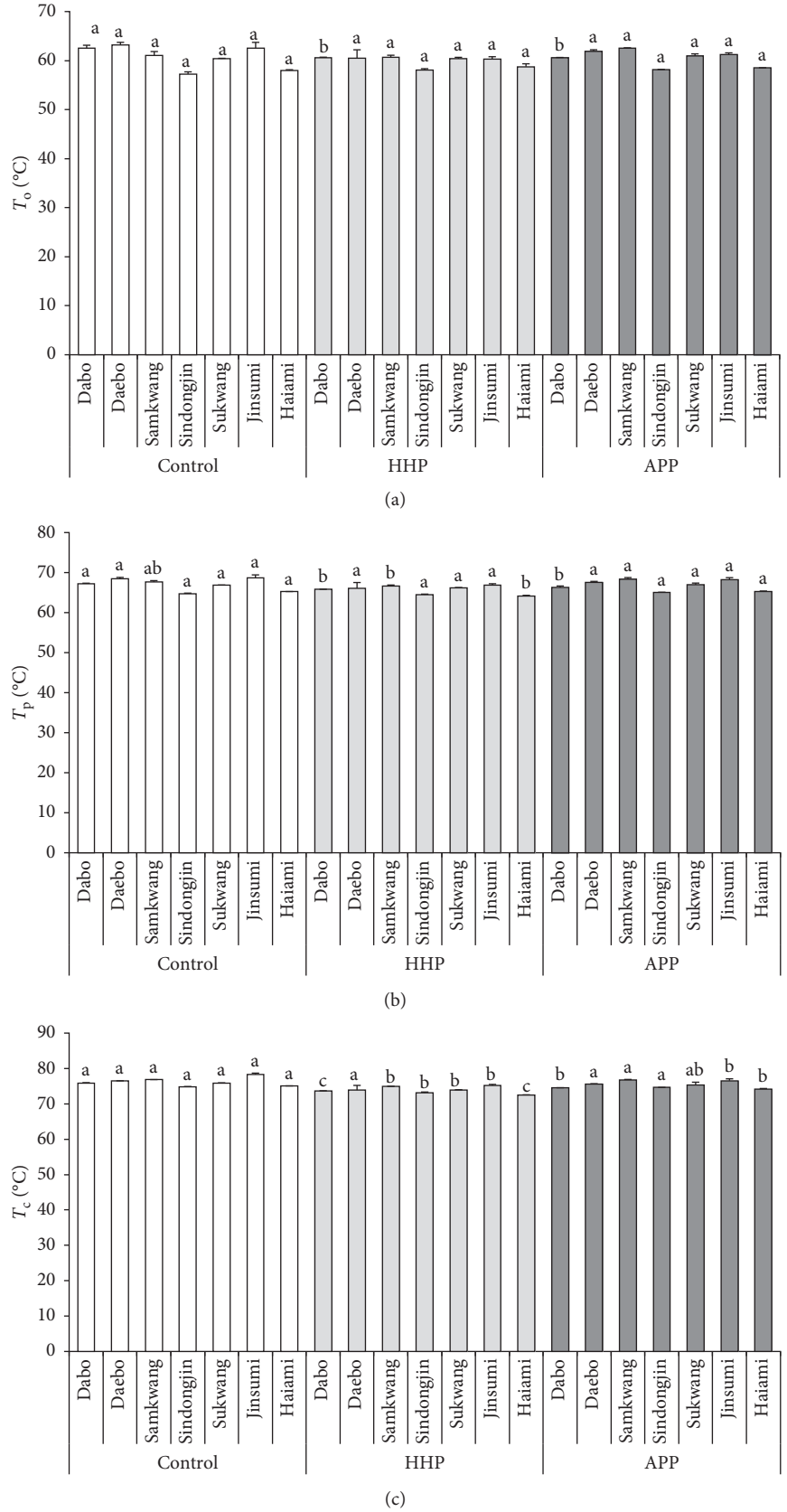


Figure 5: Continued.

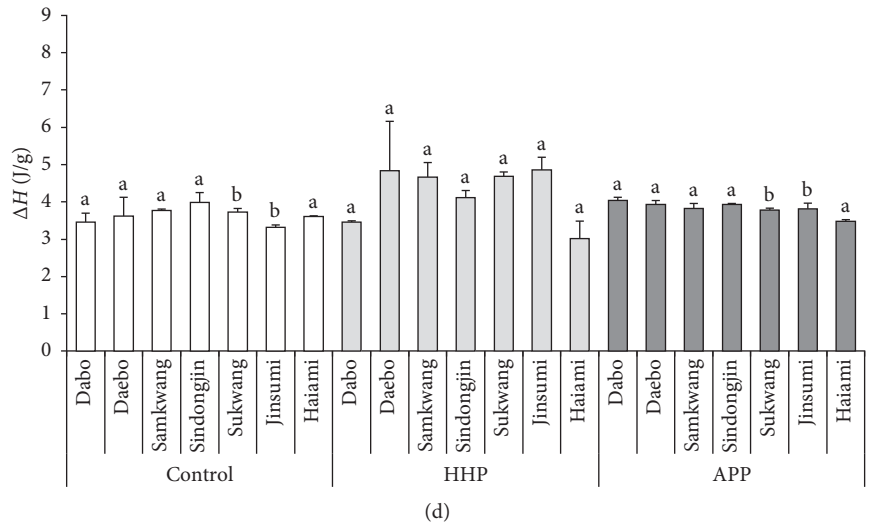


Figure 5: Differential scanning calorimetry (DSC) thermodynamic properties of rice. Samples were treated by high hydrostatic pressure or atmospheric pressure plasma, and DSC was compared to control. Values with different superscripts are significantly different at $p < 0.05$ according to Tukey's multiple range tests within the same cultivars.

APP treatment was similar to that of the control group or slightly altered in some varieties, but the change was less than that of the HHP treatment.

Overall, the color difference was highest for the *a* value after HHP treatment, and APP-treated samples were similar to the control. Previous studies that used HHP to treat food materials also showed changes in Hunter Lab values. However, the difference was less than thermal treatment because the color components are sensitive to temperature [19, 20]. Therefore, nonthermal treatments, especially APP, minimize color changes in foods with effective microbial inactivation.

3.5. DSC Thermodynamic Properties of Rice after HHP and APP Treatments. We analyzed starch retrogradation by DSC (Figure 5). The enthalpy change (dH) was determined by the energy transformation during the melting of recrystallized amylopectin, which is responsible for starch retrogradation [21]. There were no significant differences between the HHP or APP treatment groups compared to the control group, except for the HHP-treated Sukwang and Jinsumi cultivars (26% and 46% increases, respectively). The onset temperature (T_o), maximum peak temperature (T_p), and completion temperature (T_c) are dependent on the structure or degree of hydrogen bonding of the starch. These values predict the melting and destruction points of the amylose complex [21]. T_o value was decreased with HHP and APP treatment only in the Dabo variety. T_p values were significantly decreased in Dabo after HHP and APP and in Haiami after HHP. On the other hand, T_c was decreased significantly by HHP treatment (except for Daebo) and by APP treatment in Dabo, Jinsumi, and Haiami.

Starch retrogradation may occur following gelatinization, and HHP has been reported to induce damage to the starch structure by initiating gelatinization. The degree of retrogradation following gelatinization is lower in HHP than in thermal treatment due to the lower moisture content [22]. APP, which works by reactive energetic electrons, sometimes affects the inner portion of cereals, which results in damage to the interior organization of the grains [23]. However, the HHP and APP treatment procedures used in this study did not markedly alter the retrogradation factors of the rice. This may be due to different experimental factors such as time, pressure, and plasma intensity. Therefore, this result provides appropriate methods for microbial inactivation in rice with only minor changes to retrogradation factors.

In conclusion, The HHP and APP treatments effectively controlled the microbial population of the rice cultivars in this study. In particular, HHP powerfully sterilized microbial growth and produced remarkable changes in the physicochemical properties of the rice (pH, brightness, *a* value, and free sugar composition). In contrast, APP showed only a mild effect on rice qualities with an ~34% reduction in the microbial population. The results of this study could be applied in the rice processing industry and may provide a suitable method for both microbial regulation and maintenance of rice quality. The application of either HHP or APP could be considered depending on the intended use of the rice.

Abbreviations

APP: Atmospheric pressure plasma
CFU: Colony-forming unit
DBD: Dielectric barrier discharge
DSC: Differential scanning calorimetry
HHP: High hydrostatic pressure
HPLC: High-performance liquid chromatography.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Microbial Decontamination of Onion by Corona Discharge Air Plasma during Cold Storage

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Corona discharge air plasma (CDAP) is a nonthermal decontamination technology which is generating antimicrobial agents such as photons, electrons, positively and negatively charged ions, atoms, and free radicals. We investigated the effect of a corona discharge under atmospheric pressure on the sterilization of postharvest fungal pathogens on onion. The main antimicrobial reactive substance generated by CDAP was O_3. The active species such as nitric oxide (NO) and nitric dioxide (NO_2) were nearly detected in this experiment. CDAP treatment revealed different isolation frequencies depending on postharvest pathogens from diseased onions, showing less isolation frequency of *Fusarium* spp. and *Alternaria* sp. than that of *Botrytis* spp. when compared with untreatment onions during 10-month cold storage. CDAP treatment at 2~2.6 ppm of O_3 slightly stimulated the mycelial growth of *Alternaria* sp., while the treatment at 20~24 ppm of O_3 gradually inhibited mycelial growth by treatment time. However, *Botrytis* sp. showed different patterns of mycelial growth with CDAP treatment. Less than 4 hours' treatment of CDAP slightly inhibited the mycelial growth of *Botrytis* sp., while 8 hours' treatment of CDAP slightly stimulated the mycelial growth of *Botrytis* sp. not depending on the concentration of O_3. The inhibitory effect of CDAP on the conidial germination of *Alternaria* sp. and *Botrytis* sp. was examined with treatment time and intensity of CDAP. The conidial germination of *Alternaria* sp. treated with CDAP at the concentration of 13.7~14.4 ppm of O_3 was strongly inhibited by time, showing $y = 2.66x^2 - 85.139x + 4.88$ and $R^2 = 0.98$. When the conidia of *Alternaria* sp. were exposed for 2 hours with varying plasma O_3 concentration, the conidial germination was strongly inhibited as the concentration of O_3 increases, showing $y = -0.09x^2 + 6.905x - 0.764$ and $R^2 = 0.95$. The conidia of *Botrytis* sp. also showed similar patterns to CDAP. The inhibitory effect of CDAP on the germination of postharvest pathogens depends on treatment time and O_3 concentration.

1. Introduction

Onion, one of the widely consumed vegetables, is well known for various biological activities including antioxidant and antibacterial effects mediated by sulfur and phenolic compounds [1, 2]. Onion is commonly used as a spice in Korea. Onion is usually stored for several months in a cold, dry condition after curing process to cover the seasonal demands of market in Korea. Despite the cold storage to keep marketable quality, the onion losses are substantially occurred during the storage. The major losses are caused by plant pathogens without appropriate management of postharvest diseases [3]. *Botrytis* sp., *Fusarium oxysporum*, *Penicillium* sp., *Aspergillus awamori*, *Rhizopus oryzae*, and *Alternaria* sp. are well known to cause decay during onion storage in Korea [4, 5]. However, the application of agrochemical fungicides is limited because of public concerns over the human health and environmental risks over the agrochemical residues. Therefore, eco-friendly alternative measures should be considered to control the postharvest pathogens contaminated on onions bulbs.

Plasma is known as the state of ionized gas which contains energetic reactive species, such as electrons, photons, ions, free radicals, excited molecules, and atoms, and is considered as an emerging technology for the management of postharvest diseases. There are several methods to generate plasma, including gas discharge, photoionization, heat radiation, and radio frequencies. Among the methods, the common way to produce nonthermal plasma is gas discharge [6]. Corona discharge air plasma (CDAP) and dielectric barrier discharge are the most common approaches for nonthermal plasmas' generation under atmospheric pressure. They are known to produce chemically active species, oxygen ions, and charged species such as NO+, NO−, hydroxyl and hydroperoxyl radicals, hydrogen peroxide, nitrogen oxide species (NO, NO_2, etc.), atomic oxygen, and ozone [7]. The gases widely used to create plasma are air, pure Ar, mixture of He/O_2 and Ar/O_2, and pure N_2 [8]. These active species act as very strong oxidizers and are considered to contribute to the antimicrobial effects of gas plasma [9]. There are many reports that state that low-temperature atmospheric plasma can kill various kinds of microorganisms, such as fungi, bacteria, and yeast [10, 11]. The mechanisms of nonthermal plasma for the inactivation of microorganisms are suggested as surface erosion and oxidation of microbial cell membranes by reactive species [12] and DNA damage by UV radiation [13]. The potential of cold atmospheric plasmas for antimicrobial applications has been reported earlier. One atmosphere uniform glow discharge plasma has been used for the successful inactivation of *Escherichia coli* O157:H7, *Salmonella* sp., and *Listeria monocytogenes* on fresh produce surfaces [14]. As it constitutes one of the forms of atmospheric plasma, corona discharge plasma has also been shown to possess biocidal or biodecontamination effect [15, 16]. The predominant mechanism of biological action of corona discharges is believed to be oxidative damage produced by reactive oxygen species [17]. However, the effect of plasma sterilization depends on the kind of microorganisms, initial population of contaminated microorganisms, plasma treatment temperature, and relative humidity [18]. Sera and Sery [19] reported that the major sterilization factors in the nonthermal plasma food technology sector largely depend on the plasma source type or plasma characteristics. They reported the availability of nonthermal plasma for activation of seed germination, early growth of seedlings, microbial inactivation of seed/fruit surface, and possibility of increasing quantity of biological active compounds in sprouting seeds. In general, fungi are more profound than bacteria or viruses and have cell walls that lead to less susceptibility to external cell damage. In this study, we examined the effects of CDAP for the inactivation of postharvest pathogens contaminated on onion and investigated the inhibitory effect of CDAP on the mycelial growth and conidial germination of *Alternaria* sp. and *Botrytis* sp.

2. Materials and Methods

2.1. Corona Discharge Air Plasma Generation. Corona discharge air plasma (CDAP) used in this experiment was purchased from Samdo Environment Co. Ltd., Kwangju, Korea. A schematic diagram of CDAP is shown in Figure 1. An air blower (Ventur Tekniska, Goteborg, Sweden) to generate remote or afterglow plasma stream from electrode point had 25 lpm of blower rate at the electrode tip. Power supply with the output voltage of 20 kV DC and the frequency of 60 Hz was used for the plasma. Plasma intensity was controlled by adjusting the electric current and frequency. The amounts of ionized gas of the plasma were determined by using a plasma-activated species (O_3, NO, and NO_2) detector. The amount of active species produced is shown in Figure 2 and Table 1, respectively. Major active species of corona discharge air plasma was ozone. NO_2 was poorly generated, and NO was not generated. The ionized gas of the plasma was flowed by channeling through a PVC flexible hose (1 m length; 70 mm in diameter) to a treatment chamber (0.35 m^3 in dimension) or generated in a cold storage room (50.4 m^3 in dimension).

2.2. Isolation and Identification of Postharvest Pathogens of Onion. To investigate the effect of CDAP on onion decay in low-temperature storage conditions, onion was stored at 0°C in a cold storage room (50.4 m^3 in dimension) for 10 months with corona discharge air plasma treatment at O_3 concentration of 5 ppm for 6 hours every day. The decayed onions were selected as plasma treated or untreated one after 10 months' storage. To isolate the causal fungi responsible for onion decay, the tissue (10 × 10 mm) of the boundary between the healthy and diseased areas on the decayed onion was aseptically taken. The tissues were sterilized with 70% ethanol and 1% NaOCl solution for 1 min., respectively. The tissue was washed with sterile distilled water and then dried on a sterile filter paper. Then, it was placed on a prepared water agar plate and incubated at 25°C. The grown mycelium was aseptically transferred to potato dextrose agar (PDA) and examined for single isolate under a microscope. The morphological characteristics of each isolated fungus were observed under an optical microscope. The frequency of isolated fungi was calculated by counting the number of individual pathogens among the total isolated.

2.3. Pathogenicity Test of the Isolates. Each isolate was cultured on PDA for 7 days and used for the pathogenicity test. The onion kept in the 0°C low-temperature storage was selected, and the skin and roots were removed. Then, the onion was washed with clean water and dried for 4 hours. Then, the onion was cut to half with a sterilized knife by an alcohol lamp and 70% ethanol. One side of the onion was injured with a needle, and the other side was prepared without injury. A wet paper towel with sterilized water was placed on the bottom of a plastic box (200 × 280 × 200 mm), and clean Petri dishes (35 × 10 mm; SPL Life Science Co., Pocheon, Korea) were placed on the paper towel. Six half onions (3 noninjured and 3 injured) were placed in Petri dishes on the bottom of a plastic box. Each isolate prepared to a Φ10 mm-sized mycelial disk was inoculated on the onion. Then, the plastic box was stored at 25°C to determine

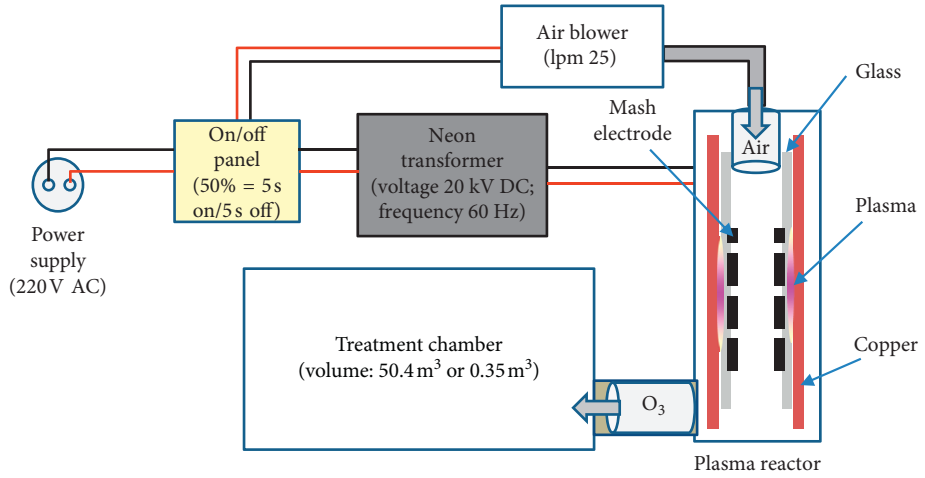

Figure 1: Schematic diagram of corona discharge air plasma (CDAP) system. 50.4 m^3 is the generated plasma in a low-temperature storage room. 0.35 m^3 is the scale of the device installed to check the degree of the inhibition of the microbial isolated from the onion.

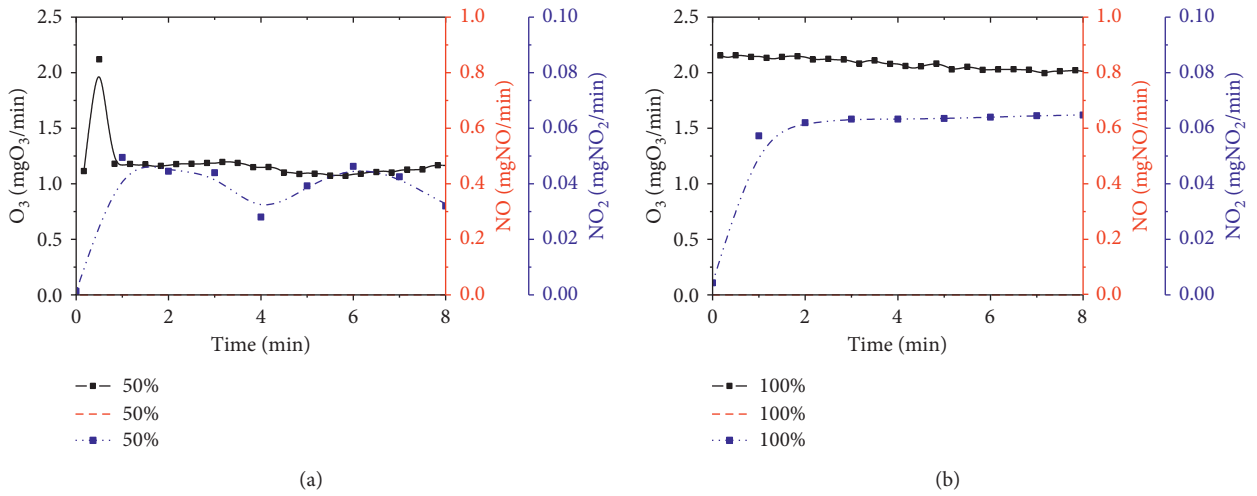

Figure 2: Active species generation amount of corona discharge air plasma.

Table 1: Measurement of active species generated by corona discharge air plasma.

	Average generation rate at 25 lpm air, 8 min		Average concentration at 25 lpm air, 8 min	
	50%	100%	50%	100%
O_3	1.18 (mg/min)	2.07 (mg/min)	22 (ppm v/v)	38.7 (ppm v/v)
NO_2	0.033 (mg/min)	0.058 (mg/min)	0.644 (ppm v/v)	1.13 (ppm v/v)
NO	0	0	0	0

whether the disease develops. All isolates were tested for pathogenicity in three replicates over the second time.

2.4. Inhibitory Effect of Corona Discharge Air Plasma on the Mycelial Growth of the Postharvest Pathogens. We investigated the inhibitory effect of plasma treatment on the mycelium growth of two pathogens, *Alternaria* sp. 5RD1 and *Botrytis* sp. 2RG4, isolated from the diseased onion. They were inoculated on the PDA medium and cultured in a 25°C incubator for 7 days. The mycelial disk of the pathogens was prepared using a cork borer (Φ10 mm) and placed on a new PDA medium. Then, the PDA plate without lid was placed in an acrylic box equipped with a plasma device and treated for a certain period of time (1, 2, 4, 8, and 16 hours). After treatment, the PDA plate was cultured at 25°C to observe the mycelial growth for 7 days. The inhibitory effect was calculated by comparing the mycelial growth of the pathogens with and without plasma treatment.

2.5. Inhibitory Effect of Corona Discharge Air Plasma on the Spore Germination of Postharvest Pathogens. Alternaria sp. and *Botrytis* sp. were inoculated on a potato dextrose agar (PDA) medium and cultured at 25°C for 10 days to form its spores. 20 ml of sterile water was added to the PDA plate, and the spores were suspended using a sterile loop. The

spore suspension (approximately 1×10^6 spores/ml) was prepared by passing through sterilized four-layered gauze to remove mycelial fragment and stored at 4°C before use. 100 μL of the spore suspension was inoculated on the surface of water agar for plasma treatment. Then, the water agar plate without lid was placed into a disinfected acrylic container connected with a plasma device by a hose. The plasma was treated at different time periods or O_3 concentration. After treatment, the plates were incubated for 16 hours at 25°C, and spore germination was observed under an optical microscope.

3. Results and Discussion

3.1. Active Species Generated by Corona Discharge Air Plasma. To measure the amount of plasma active species, the air flow of the internal suction pump was set to 25 lpm and the power setting value (time) was increased from 50% (5 s on/5 s off) to 100% (10 s on/10 s off). As shown in Figure 1 and Table 1, when O_3, NO_2, and NO detectors were used for the plasma active species, O_3 was presented for most of the active species. The average generation rate of O_3 was 1.18 mg/min at 50% (5 s on/5 s off) and 2.07 mg/min at 100% (10 s on/10 s off). The average generation rate of NO_2 was 0.033 mg/min at 50% (5 s on/5 s off) and 0.058 mg/min at 100% (10 s on/10 s off). NO was not detected. Plasma is known to vary in the ionized material produced by the process gasses used. Hertwig et al. [20] found that there was a significant difference in plasma emission intensity depending on the gas types (dry air, N_2, O_2, and CO_2). N_2 as a process gas showed the highest emission intensity compared to the other process gasses. They also reported that the use of O_2 as a flower gas with cold atmospheric pressure plasma produced a high ozone concentration in the treatment chamber. We also achieved a similar result that relatively high ozone concentration was detected with the use of the air as a flower gas of CDAP.

3.2. Types of Fungi Isolated from Decayed Onions. The decayed onions were separated as untreated or CDAP-treated one after 10-month storage at 0°C, and the causal agents were isolated. As a result of the isolates in Table 2 and Figure 3, a total of 77 fungi were isolated from CDAP-treated onion and total 103 molds from nontreated onion. When the fungi were classified by type, more *Fusarium* sp. were isolated from nontreated onions and more *Botrytis* sp. were isolated from CDAP-treated onions. The frequency of isolation by fungal type showed 24% of *Botrytis* sp., 70% of *Fusarium* sp., 2.0% of *Alternaria* sp., and 7.0% of unknown fungi in untreated (control) onions. On the other hand, it showed 39% of *Botrytis* sp., 26% of *Fusarium* sp., 0% of *Alternaria* sp., and 12% of unknown fungi in CDAP-treated onions. In general, *Fusarium* sp. is the dominant strain at 25°C, and *Botrytis* sp. is known to occur well below 15°C. Our results from Figure 2 suggest that the major active species on the antimicrobial effect of the CDAP-treated onion is ozone. Our result suggested that ozone seems to be more critical to the growth of *Fusarium* sp. than the other fungi isolated in this experiment. Ozone is known as a powerful oxidant and has been researched as a sanitizer in the food industry [21] and a removal agent of mycotoxins [22] or pesticide residues [23]. Ozone can also act as a host resistant inducer. Minas et al. [24] reported that the exposure of kiwifruits to ozone before inoculation of *Botrytis cinerea* in a cold storage room resulted in the reduction of disease incidence. They suggested that the treatment of ozone to kiwifruit induces resistance to *B. cinerea*.

3.3. Pathogenicity and Characteristics of Fungi Isolated from Decayed Onions. As a result of testing pathogenicity of isolates in Table 3, *Fusarium* sp. 3RC2 and *Fusarium* sp. 3RC1 revealed as strong pathogenic fungi on both wound and healthy onion. In addition, *Alternaria* sp. 5RD1 showed strong pathogenicity on wounded onion but weak pathogenicity on healthy onion. The unidentified fungus 2RC1 showed strong pathogenicity only in wounded onion. *Botrytis* sp. 2RG4 and 3RA2 and unidentified fungus 3RB2 showed intermediate pathogenicity only in wounded onion. The isolated fungi are mostly spore-forming fungi, which may be rapidly decayed by spore's germination during storage and distribution.

3.4. Inhibitory Effect of Plasma on the Mycelial Growth and Spore Germination of Isolates. We investigated whether CDAP treatment by time and intensity inhibits the mycelial growth of *Alternaria* sp. 5RD1 and *Botrytis* sp. 3RG4 on the PDA medium. Treatment of CDAP at 10% intensity stimulated the mycelial growth of the isolate, showing the mycelial growth rate of −3.36% in 1 hour, −7.21% in 4 hours, and −2.51% in 16 hours, respectively. Treatment of CDAP at 50% intensity showed the mycelia growth rate of −4.33% in 1 hour, −1.44% in 4 hours, and 5.86% in 16 hours. The higher the concentration of the plasma treatment or the longer the plasma treatment time, the more inhibitory effect on the mycelial growth of *Alternaria* sp. 5RD1 was observed (Figure 4(a)).

CDAP treatment to *Botrytis* sp. 3RG4 inhibited the mycelial growth up to 6.57% in 1 hour, 10.22% in 2 hours, and 7.66% in 4 hours at 10% plasma intensity, but treatment of CDAP with increased time slightly stimulated the mycelial growth of *Botrytis* sp. 3RG4, showing the mycelial growth rate of −3.65% and −5.15% in 8 hours and 16 hours, respectively. Treatment of CDAP at 50% intensity showed a similar mycelial growth pattern with 10% plasma intensity (Figure 4(b)). Our results showed that the CDAP treatment effect on the mycelial growth of *Alternaria* sp. 5RD1 and *Botrytis* sp. 3RG4 isolated from decayed onions is little significant or insignificant.

We investigated whether CDAP treated by time and plasma intensity influences on the spore germination of *Alternaria* sp. 5RD1 and *Botrytis* sp. 3RG4. The inhibitory effect of CDAP at 45% intensity (O_3 concentration: 13.7~14.4 ppm) on the spore germination of *Alternaria* sp. 5RD1 is shown in Figure 5. The inhibition rate of spore germination of the isolate was 72.6% for 1 hour and 92.3% for 2-hours exposure. Regression analysis showed high

Table 2: Comparison of isolation frequency of fungi isolated from nontreated or CDAP-treated onions.

Treatment	Frequency of isolation (%)				Total isolates
	Botrytis spp.	*Fusarium* spp.	*Alternaria* spp.	Unknown	
Control	24	70	2.0	7.0	103
CDAP	39	26	0	12	77

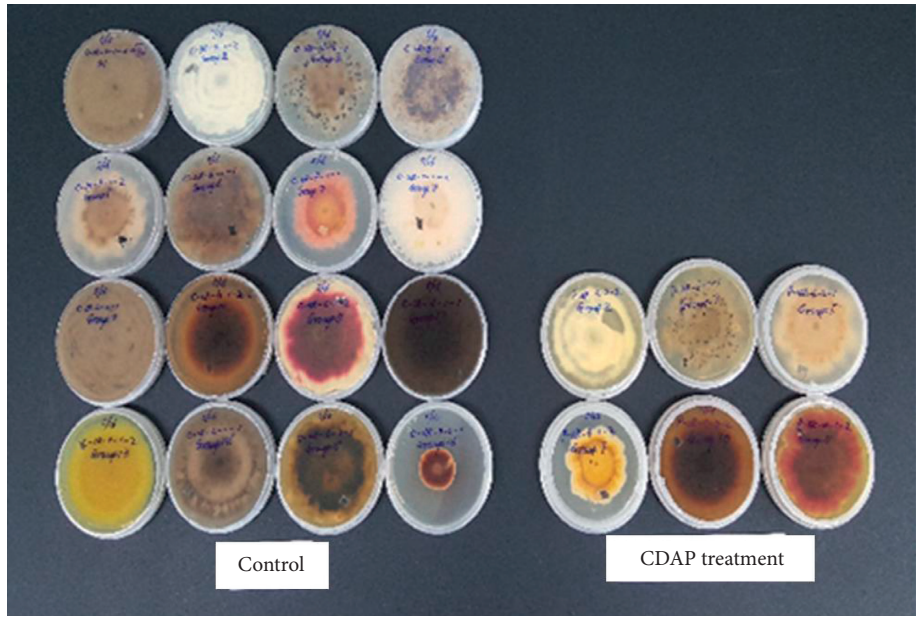

Figure 3: Colors of representative fungal isolates grown on potato dextrose agar isolated from nontreated or CDAP-treated onions.

Table 3: Pathogenicity and characteristics of fungi isolated from decayed onions.

Strains	Pathogenicity		Characteristics in potato dextrose agar
	Injured onion	Fresh onion	
Fusarium 3RC1	+++	++	White mycelium, rod-shaped conidiospore, good mycelium growth
Fusarium 3RC2	+++	+++	White mycelium, rod-shaped conidiospore, good mycelium growth
Alternaria 5RD1	+++	+	Conidiospore, medium mycelium growth
Unknown 2RC1	+++	−	Nonspore formation, poor mycelium growth
Botrytis 2RG4	++	−	Small-type conidiospore, good mycelium growth
Botrytis 3RA2	++	−	Grey mycelium, small-type conidiospore, good mycelium growth
Unknown 3RB2	++	−	Nonspore formation, medium mycelium growth
Botrytis 1RA2	+	−	Grey mycelium, small-type conidiospore, poor mycelium growth
Unknown P2RB1	+	−	Nonspore formation, poor mycelium growth
Unknown P5RF2	+	−	Nonspore formation, poor mycelium growth

+++ and ++: good mycelium growth; +: medium mycelium growth; −: poor mycelium growth.

significance at $y = 2.66x^2 - 85.139x + 4.88$ and $R^2 = 0.98$ (Figure 5(a)). When *Alternaria* spores were exposed with various intensities (various ozone concentrations) of CDAP for 2 hours exposure, the inhibition rate of spore germination was 21.7% for ozone concentration of 4.37 ppm, 72.7% for 10.85 ppm, and 95.41% for 19.45 ppm. Regression analysis showed high significance at $y = -0.09x^2 + 6.905x - 0.764$ and $R^2 = 0.95$ (Figure 5(b)).

The inhibitory effect of CDAP at 10% intensity (O_3 concentration: 1.5~2.9 ppm) on the spore germination of

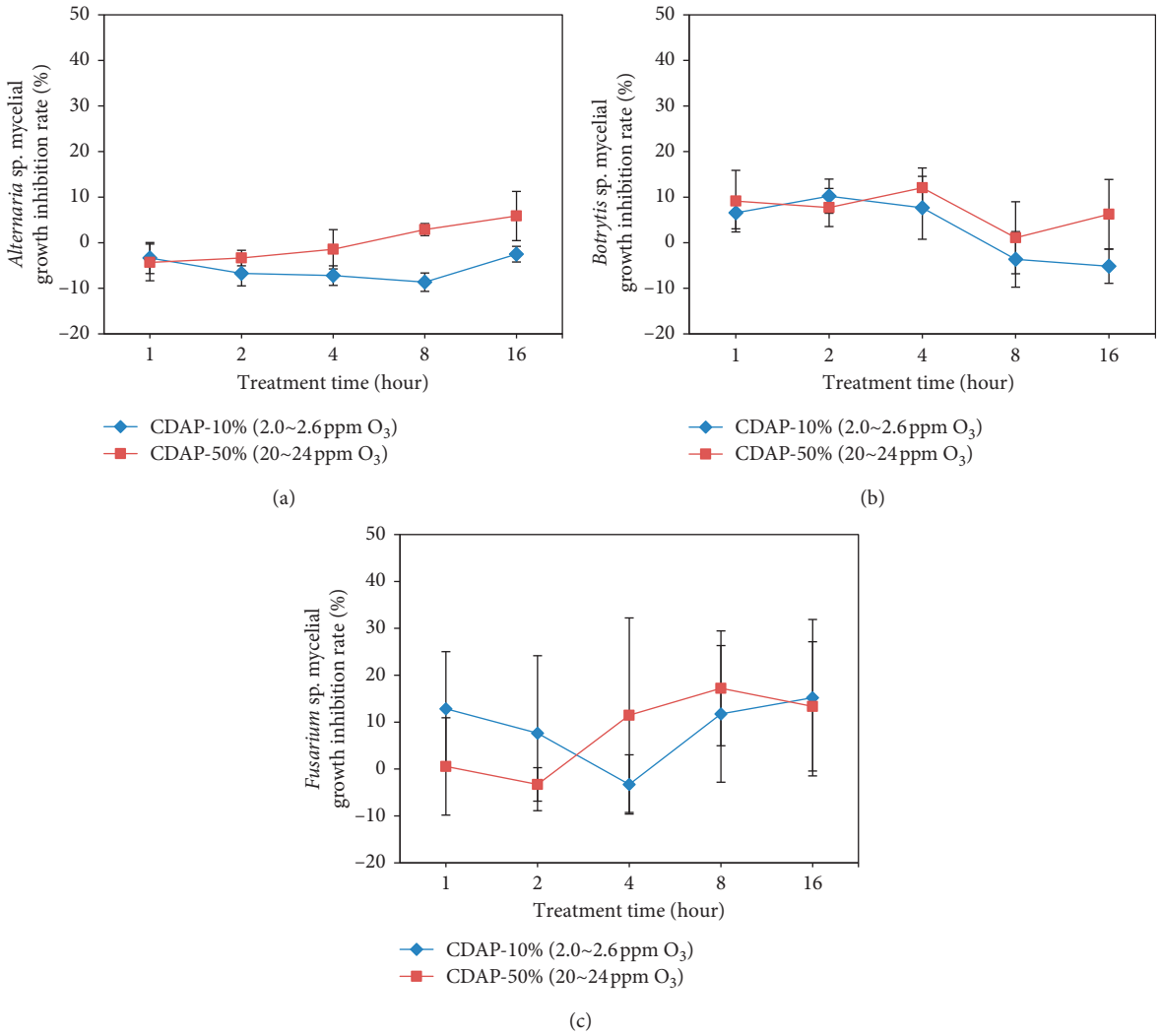

Figure 4: Effect of CDAP treatment time on mycelial growth of *Alternaria* and *Botrytis* sp. (a) Mycelial growth of *Alternaria* sp. 5RD1 after culturing at 25°C for 4 days after CDAP treatment, (b) mycelial growth of *Botrytis* sp. 2RG4 after culturing at 25°C for 3 days after CDAP treatment, and (c) mycelial growth of *Fusarium* sp. 3RC2 after culturing at 25°C for 3 days after CDAP treatment. The CDAP-10% and CDAP-50% mean that the plasma operation time is operation on during 1 second/operation off during 1 second (concentration of O_3 is 2.0~2.6 ppm) at CDAP-10% and operation on during 5 second/operation off during 5 second (concentration of O_3 is 20~24 ppm) at CDAP-50% per hour for 16 hours.

Botrytis sp. 3RG4 is shown in Figure 6. The inhibition rate of spore germination was 70.7% for 4 hours and 98.5% for 8 hours of exposure, respectively. Regression analysis showed high significance at $y = -0.684x^2 + 18.307x - 4.809$ and $R^2 = 0.95$ (Figure 6(a)). When the spore of *Botrytis* sp. 3RG4 was treated with various intensities (various ozone concentrations) of CDAP for 2 hours exposure, the inhibition rate of spore germination was 23.7% for ozone concentration of 2.1 ppm, 56.9% for 3.39 ppm, and 97.53% for 6.0 ppm, respectively. Regression analysis showed high significance at $y = -0.635x^2 + 19.347x - 4.772$ and $R^2 = 0.97$ (Figure 6(b)).

The inhibitory effect of fungal spores in Figures 4–6 was much better than that of mycelium. The inhibitory effect of fungus mycelium on ozone produced in the CDAP treatment was not effective in *Fusarium* sp. (Figure 4(c)). *Alternaria* sp. and *Botrytis cinerea* showed some inhibitory effect at high O_3 concentration, but it promoted the growth of fungus mycelium at low O_3 concentration (Figures 4(a) and 4(b)). However, the inhibitory effect of fungi spores on ozone showed more than 80% inhibition rate at ozone concentration of 13~14 ppm with 2 hours treatment in *Alternaria* sp. spores (Figure 6(b)) isolated from onion and at ozone concentration of 6 ppm with 2 hours treatment for *Botrytis cinerea* spores (Figure 5(b)). These results indicate that the effect of plasma treatment varies depending on the kind of fungal species and propagules. Gabler et al. [25] also reported that ozone treatment effectively controlled the postharvest grey mold disease on grapes, while poorly controlled the decay of grapes caused by *Alternaria* and *Penicillium* sp. in semicommercial experiments. Minas et al. [24] reported that continuous treatment of *B. cinerea* cultures grown on potato dextrose agar with gaseous ozone showed a direct inhibitory effect, but removal of the pathogen from the ozone-enriched environment led to resume the growth within 48 hours. They also

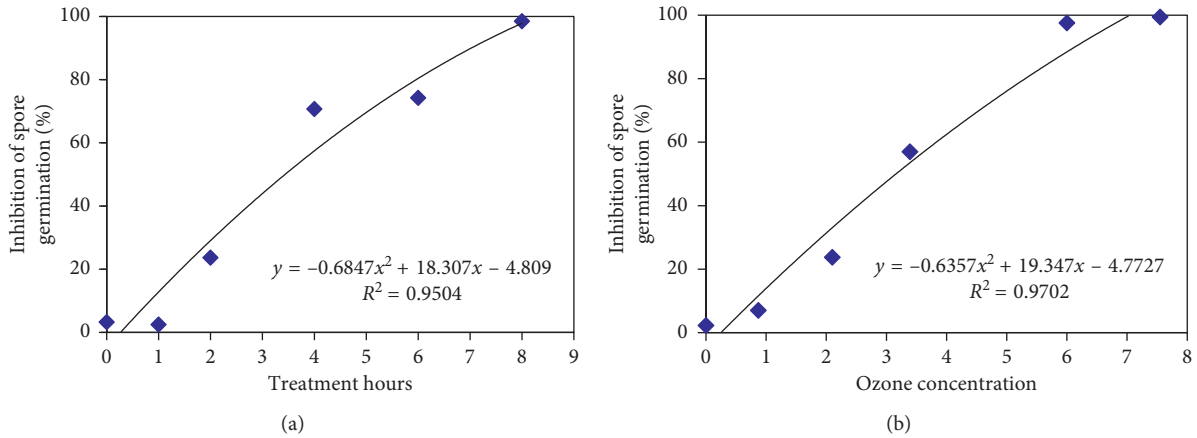

Figure 5: The effect of different plasma treatment time and concentration on the inhibition of germination of *Botrytis sp.* spore. (a) Plasma treatment of *Botrytis sp.* spores with different treatment time periods at constant O_3 concentration (CDAP-10%, O_3: 1.5~2.9 ppm) and (b) plasma treatment of *Botrytis sp.* spores with different O_3 concentrations at constant treatment time (2 hours).

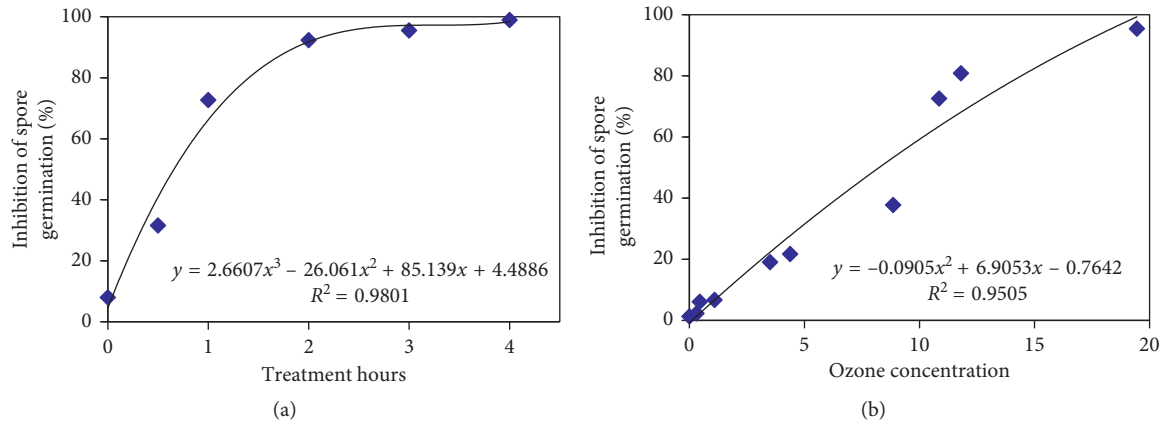

Figure 6: The effect of different plasma treatment time and concentration on the inhibition of germination of *Alternaria sp.* spore. (a) Plasma treatment of *Alternaria sp.* spores with different treatment time at constant O_3 concentration (O_3: 13.7–14.4 ppm) and (b) plasma treatment of *Alternaria sp.* spores with different O_3 concentrations at constant treatment time (2 hours).

reported that gaseous ozone treatment of the fungal spores for more than 8 hours significantly reduced the spores' viability. Ryu et al. [26] measured the extent of spore survival and the shape of spores by treating plasma with spore of the bread mold. The fungus has less sterile effect by the plasma treatment than the bacteria, because the fungus has a cell wall composed of a carbohydrate called β-glucan. In the case of bread molds, β-carotene, which acts as an antioxidant, is present in large amounts in the spores and may have the effect of protecting it from oxidation by plasma. Many molds have a variety of pigments, and these pigments can also act as antioxidants in many cases, so that, the fungus may be more resistant than bacteria to plasma treatment. Our data in this study showed that CDAP treatment significantly triggered fungal spore death depending on time. These data suggest that CDAP is a promising tool to inactivate the fungal spores. However, the optimization of CDAP processing conditions should be evaluated by the kind of microorganisms, type of products, temperature and humidity in a storage, etc.

4. Conclusions

The main antimicrobial reactive substance generated by the CDAP method was ozone. Ozone produced by CDAP treatment may be effective in inactivating fungal spores, whereas inactivation of fungal mycelium was not effective. Data presented in this report demonstrated that the low concentration exposure of CDAP had the potential of inactivating fungal spores. In addition, CDAP treatment triggered significantly fungal spore death that depends on time. These data suggested that plasma represents a novel technology with the capacity of inactivating fungal spore in plants. The inhibitory effect of CDAP on the germination of postharvest pathogens depends on treatment time and O_3 concentration.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Y.S.B. and H.J.C. conceived and designed the study; E.H.C. and Y.S.B. carried out the experiments; I.S.S., J.H.L., and J. W.C. analysed the data; and E.H.C. and Y.S.B. wrote the manuscript. All authors read and approved the final manuscript.

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