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Broccoli: Antimicrobial Efficacy and Influences to Sensory and Storage Properties by Microwave Plasma-Processed Air Treatment

Uta Schnabel,[a,*] Rijana Niquet,[a] Mathias Andrasch,[a] Marion Jakobs,[b] Oliver Schlüter,[c] Kai-Uwe Katroschan,[b] Klaus-Deter Weltmann,[a] & Jörg Ehlbeck[a]

[a]Leibniz Institute for Plasma Science and Technology, 17489 Greifswald, Germany; [b]Landesforschungsanstalt für Landwirtschaft und Fischerei M-V, 18276 Gülzow, Germany; [c]Leibniz Institute for Agricultural Engineering and Bioeconomy, 14469 Potsdam, Germany

*Address all correspondence to: Uta Schnabel, Leibniz Institute for Plasma Science and Technology, Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany, E-mail: uta.schnabel@inp-greifswald.de

ABSTRACT: Currently used disinfection and sanitation methods for fresh fruits and vegetables lack antimicrobial effectiveness and are high in cost, water consumption, or chemicals. One alternative may be nonthermal plasma at atmospheric pressure. The plasma setup used depends on microwave plasma, which generates plasma-processed air (PPA) with manifold chemical and antimicrobial compounds mainly based on reactive nitrogen species. Fresh broccoli florets were contaminated with seven different microorganisms (bacteria, yeast, and endospores) and then treated with PPA. After a maximum treatment time of 15 min, reduction rates greater than 5 log were achieved. Furthermore, sensory examination and storage experiments showed influences on texture, appearance, odor, and shelf-life. Clearly, plasma and the generated chemical mixture that leads to high microbial inactivation on specimens offer a wide range of possible uses. However, food quality must be further investigated.

KEY WORDS: antimicrobial effects, broccoli, microwave plasma, sensory properties, shelf-life, storage

I. INTRODUCTION

Fresh produce has a limited shelf-life of several days, which allows for only distribution. There are various causes for this limited shelf-life and the associated losses of fresh produce, but all depend on microbial contamination at all stages in the value chain. Microbial contamination may also cause foodborne illnesses worldwide.

Broccoli (*Brassica oleracea* var. *italica*) is commonly converted to fresh-cut floret products and the stems can also be shredded into packaged coleslaw-type products. At cultivation, the prediction of harvest date and therefore the management of harvest amount are difficult. The storage of packaged and marketable broccoli is typically after the peak to guarantee the continuous supply of the food retail industry despite fluctuating yields. Field-packed broccoli is commonly cooled by injecting liquid ice into waxed cartons.[1] The ice maintains the low temperature and relative humidity for transport and

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distribution. If storage of broccoli is necessary, it can be kept for 2–3 weeks in the proper storage conditions (0°C, 98–100% relative humidity).[2,3]

For packaging broccoli, perforated plastic film is used to minimize product degeneration during prolonged storage, including wilting, yellowing of buds and leaves, loosening or opening of buds, and decay.[4] However, optimal controlled atmosphere conditions (1–2% O_2 and 5–10% CO_2 at 0–5°C) can double storage life.[5] A decrease of O_2 below 1% may cause off-odors due to the generation of sulfur-containing volatiles.[6]

Apart from physiological disorders such as yellow beads in overly mature broccoli and black speck on stems, which ends the commercial marketability, postharvest pathology by microorganisms are a common reason for storage losses.[1,7–11]

For shipped and stored broccoli, diseases caused by *Botrytis cinerea*, *Pectobacterium carotovorum*, and *Pseudomonas* spp. are reported. These rots can also occur at cold storage conditions due to the growing properties of these microorganisms even at very low temperatures.

Fungal diseases of broccoli include *Alternaria,* blackleg, clubroot, downy mildew, ringspot, light-leaf spot, and *Sclerotinia* spp. In addition, damping-off problems can be caused by *Pythium, Rhizoctonia,* and *Fusarium* spp. Bacterial diseases include black rot, broccoli head rot and head stem rot. Two viral diseases, cauliflower mosaic and turnip mosaic also can infect broccoli and cause production losses.[12]

Bacterial head rot of broccoli, which also occurs during storage, leads to extensive conditioning or even immense postharvest crop losses. The reason for head rot, an opportunistic disease, is a field infection of broccoli with the phytopathogens *Pseudomonas fluorescens*, *Pseudomonas marginalis*, or with *P. carotovorum*.[13] Disease incidence and severity increase when maturity coincides with periods of persistent wet weather. Storage conditions and storage time also influence disease incidence and severity. For bacterial head rot, there will not be any fungal growth unless secondary molds colonize and cause further decay. The second type of head rot is *Alternaria* head rot. *Alternaria* readily produces dark green spores on the diseased head tissue. Secondary molds and bacteria cause further decay.[14]

Current treatment options for head rot are limited to microcidal chemicals, principally those containing copper oxychloride (e.g., Cuprokylt). Treatments are generally applied as a prophylactic when the weather conditions are expected to lead to high humidity in the crop.

However, continued use of copper is finite given its phytotoxicity and toxicity in the general environment. Some European countries are now limiting its use. There are few other treatments that target the bacteria and some such as antibiotics are not permitted in parts of the European Union (e.g., the United Kingdom). Furthermore, the pseudomonads in particular are especially adaptable to sublethal levels of microcides and there are reports of resistance to copper oxychloride. It is not feasible to use a microcidal agent to eliminate bacteria from crop sites because of their ubiquity. Therefore, it is necessary to find alternative treatment approaches. Such treatments include plant defense response elicitors that trigger induced resistance pathways. A range of compounds can

elicit defense pathways and, historically, elicitors are based on natural compounds such as chitosan.[15] Others mimic plant hormones (cis-jasmone) and some have been well characterized in terms of which defensive pathway they activate. Probenazole is used as a standard treatment for rice blast in Asia and greenhouse studies have suggested that BABA (DL-3-aminobutyric acid) and ASM (acibenzolar-*S*-methyl) can reduce head rot symptoms in broccoli.[16,17] A group of approved fungicides containing strobilurins also have proven elicitor activity.[18]

The development of environmentally friendly alternative disinfection and cleaning methods at the postharvest level is also important, but product compatibility, costs, environmental impact, impact on product quality, and regulatory provisions have to be taken into account.[19] One possible alternative at the postharvest stage (e.g., during storage) is nonthermal atmospheric pressure plasma. Plasmas are ionized gases with a high proportion of freely charged particles such as ions and electrons. The properties of the plasma are not only important for photometric application such as energy-saving lamps, plasma screens, and gas lasers,[20,21] they are also used for the specific design of surfaces, including material sciences, medical technology, and microsystem technology.[22,23] Furthermore, first investigations of plasma-based microbial inactivation of fresh produce have been described in literature[24] and are currently being used in a wide range of applications for decontamination.[25–27]

In this work, investigations of inactivation kinetics and sensory and storage examinations due to plasma treatment of microbiological contaminated broccoli (*Brassica oleracea* var. italica) are presented. For broccoli florets, contaminations of Gram-negative *Escherichia coli*, *P. marginalis*, and *P. carotovorum*; Gram-positive *Listeria innocua* and *Staphylococcus aureus* and *Bacillus atrophaeus* endospores; and the yeast *Candida albicans* were used as the microbiological load.

II. MATERIALS AND METHODS

A. Investigated Microorganisms and Specimen

For microbiological experiments *E. coli* K12 (NCTC 10538), *P. marginalis* (ATCC 10844), *P. carotovorum* (ATCC 15713), *L. innocua* (ATCC 33090), *S. aureus* (ATCC 6538), *B. atrophaeus* Nakamura 1989 (ATCC 9372) in its sporolated form, and *C. albicans* (ATCC 10231) were used in concentrations of 10^8 CFU/mL suspended in sterile, distilled water. The broccoli for antimicrobial investigations and sensory examinations was bought at a local market; for storage experiments, it was provided by the fruit and vegetables producers' organization EO Mecklenburger Ernte GmbH (Wittenburg, Germany).

B. Contamination of Specimens

The broccoli was cut into florets of 10–12 g and contaminated with the bacterial or fungal suspension by pipetting a 50 µL suspension with a concentration of 10^8 CFU/mL to the florets. Afterward, the broccoli was kept under sterile and cool conditions so

that the suspension could dry aseptically under laminar flow. The broccoli for sensory and storage investigations was not artificially contaminated; only natural contamination was investigated.

C. Recovery of Microbial Contamination of Broccoli

Microbial contamination or residues in the specimens was recovered by shaking the broccoli florets in 30 mL of nutrient broth for 10 min and using the surface-spread-plate count method with agar plates. The detection limit of this procedure was 30 CFU/mL. If the number of microorganisms fell below the detection limit, that is, no viable microorganisms were found, these values were set as the detection limit in the graphs.

D. Decontamination by Microwave Plasma-Processed Air (PPA)

PPA was generated using a microwave-driven atmospheric pressure plasma source and indirect, and the contaminated florets were treated with nonthermal plasma. The microwave-driven discharge setup used is shown in Fig. 1. The microwaves had a frequency of 2.45 GHz and the supply power was in the range of 1.1 kW. Accordingly, the gas temperature was about 4000 K at a gas flux of 18 standard liter per minute (slm) air. The generated PPA was introduced into a glass bottle (250 ml) to decontaminate the fresh broccoli florets located at its bottom after cooling at room temperature.[28] PPA contains

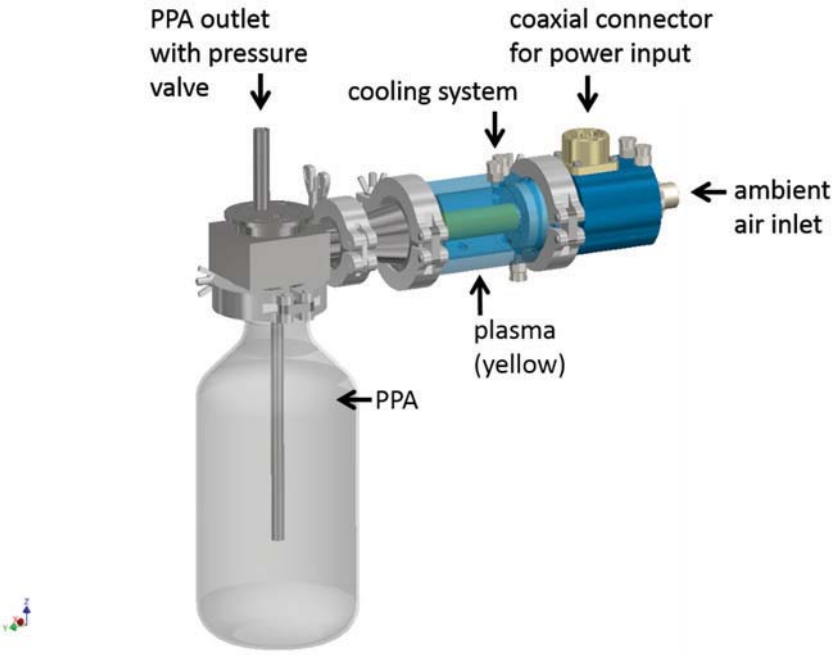

FIG. 1: Scheme of the microwave setup for the generation of PPA[31,32]

mainly reactive nitrogen species (RNS) and nonprocessed air. The RNS are used as antimicrobial agents[29,30] so that inactivation due to heat can be obviated. The discharge was ignited for 5 s (pretreatment time) and the PPA was introduced into the glass bottle. Afterward, the bottle was closed for 5, 10, and 15 min (posttreatment time) to decontaminate the inoculated broccoli florets. To stop the posttreatment time, the glass bottle was refilled twice with fresh compressed air. The observed inactivation of microorganisms depended on the amount of short- and long-lived reactive chemical species generated and storage with PPA during the post-plasma treatment time.

E. Examination of Sensory Properties

Due to the fact that the legal classification of food treatment by physical plasma remains undetermined in Germany, sensory examination and interpretation of the plasma-treated products was exclusively related to texture, appearance, and odor. The plasma-treated products were unwashed and uncut broccolis originating in Germany. The examination was done after DIN 1096 at 22°C by neu.zlt-Zentrum für Lebensmitteltechnologie Mecklenburg-Vorpommern GmbH located in Neubrandenburg, Germany. The simple descriptive test according to DIN 10964 is an analytical study and therefore an objective test in which the samples are analyzed according to certain specifications. The method is applicable for the determination of production factors and serves as a basis for the production of specific rating scales. The positive and negative features or characteristics of the test samples were described with expressions that were either arbitrary or taken from a predetermined list. Five sensory assessors examined the PPA-treated and original samples with encrypted codes. The panel did not know whether and how the broccolis were treated. After every three samples, a break was inserted and the sense of smell neutralized by coffee aroma. The broccolis were PPA treated ($n = 5$) or untreated ($n = 5$). The plasma on time was 50 s and the posttreatment time was 5 min.

F. Examination of Storage Properties

For the examination of storage properties after PPA treatment, 120 broccoli heads were investigated. These specimens were provided by the fruit and vegetables producers' organization EO Mecklenburger Ernte GmbH (Wittenburg, Germany). The investigations were done at the Landesforschungsanstalt für Landwirtschaft und Fischerei M-V (Gülzow, Germany). Parts (100 heads) of the 120 broccoli heads were short-term stored before usage. The other 20 heads were harvested directly before the examinations. The first ones were commonly produced and had a head weight of 625 g; the last ones were ecologically produced and had a head weight of 294 g. The PPA treatment of the specimens was done in IFCO boxes set in a treatment chamber of 100 L volume. To fill the treatment chamber, the microwave discharge was ignited for 50 s (pretreatment time). Different treatment scenarios were investigated (Table 1).

After PPA treatment, the broccoli was stored at 1°C and a relative humidity of 94% for 25 days. To enhance the conditions for head rot symptoms, the IFCO boxes stacked on

TABLE 1: Treatment conditions of broccoli in IFCO boxes

Sample Number	Treatment	Sample Composition
Ref	Untreated reference	2 boxes with 10 heads of 625 g and 1 box with 10 heads of 294 g
P5	5 min posttreatment PPA	3 boxes with 10 heads of 625 g
P10	10 min posttreatment PPA	3 boxes with 10 heads of 625 g
P15	15 min posttreatment PPA	2 boxes with 10 heads of 625 g and 1 box with 10 heads of 294 g

pallets were wrapped in foil. Scoring of broccoli heads for rot symptoms, grey (*B. cinerea*) and other color changes (visual), and other changes in appearance, odor, and weight was done every 2–4 days. To detect the influence of PPA treatment on shelf-life properties, the samples Ref and P5 were stored for an additional 7 days at 16°C without foil wrapping.

III. RESULTS AND DISCUSSION

The investigation of antimicrobial effects of PPA treatment of biological surfaces was based on previous work with inorganic specimens and seeds.[28,33] The optimized plasma parameters for decontamination were chosen and the treatment of broccoli took place.

A. Decontamination of Broccoli

As a biological surface, broccoli florets and heads are very complex in their surface structure and in their chemical composition. Broccoli contains many sulfur compounds with extensive health benefits. As a very healthy food, broccoli has gained new interest in science and medicine as an anticancer and antimicrobial agent and as a treatment for neurodegenerative disorder and gastric disease.

The parts of broccoli used for the microbiological experiments were single fresh florets contaminated with microbial suspensions in concentrations of 10^8 CFU/mL by pipetting. The surface drying was secured by visual inspection. All florets were treated with microwave PPA for 5, 10, and 15 min.

Experimental results (Fig. 2) showed an antimicrobial reduction of up to 5.8 log-steps depending on the microorganism. As expected, differences in the antimicrobial efficiency (kinetics) for Gram-negatives, Gram-positives, bacterial spores, and yeast were observed.

A strong decrease to at or near the detection limit after 5 min of posttreatment with PPA was seen for the Gram-negative bacteria *P. carotovorum* and the Gram-positive bacteria *S. aureus*. In the case of *S. aureus*, this reduction continued down to the detection limit after 10 min of posttreatment time. A maximum reduction of 5.8 log-steps was found. Other microorganisms, such as the bacteria *E. coli*, the yeast *C. albicans*, and the endospores of *B. atrophaeus*, needed more posttreatment time for higher reduction levels. The most difficult microorganism to inactivate were the endospores of *B. atrophaeus*, in which the maximum reduction was 2.7 log-steps after 15 min of posttreatment with PPA. With a final reduction rate of 4.7 and 4.3 log-steps, respectively, *E. coli*

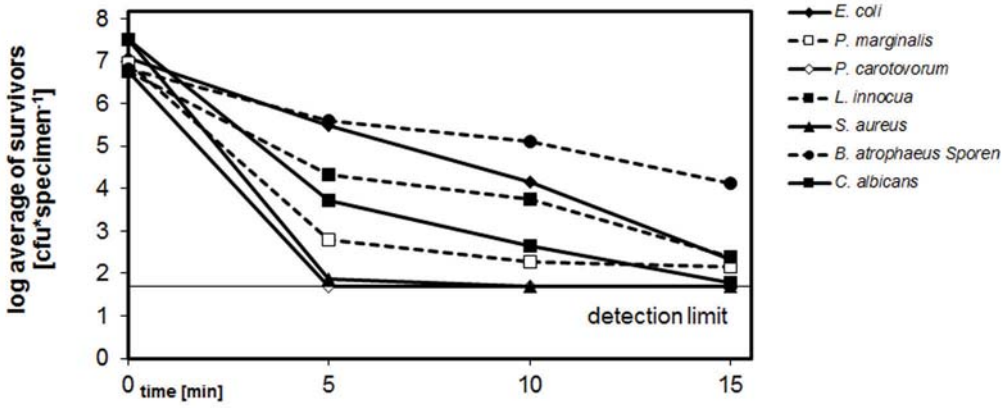

FIG. 2: Results of the posttreatment PPA of broccoli florets. Ten to 12 g of florets were contaminated with seven different microorganisms in concentrations of 10^8 CFU/mL. After a plasma ignition of 5 s, florets were incubated with microwave PPA for durations of 5, 10, and 15 min. The average of three experiments is shown. Experiments were done with $n = 3$.

and *L. innocua* showed medium inactivation consequences. The detection of *C. albicans* reached after 15 min of PPA treatment was also the limit, with 5.7 log-steps, and *P. marganilis* nearly dropped down to the detection limit, at 5.2 log-steps. For almost all investigated microorganisms, the inactivation curves showed more or less linear behavior and homogeneous kinetics. Noncontaminated broccoli florets were used as a negative reference and showed no occurrence of natural microorganisms.

Two of the most important phytopathogens connected to head rot, and thus shipping and storage losses, were investigated in these experiments. For both *P. carotovorum* and *P. marginalis*, very high reduction rates were obtained. Therefore, it is clear that PPA is a promising tool for broccoli head decontamination. The antimicrobial effectiveness is connected to the effects of RNS (e.g., NO and NO_2) on the cell walls of the bacteria.

B. Sensory Results

We also investigated whether using attributes for odor and appearance to distinguish between treated and untreated samples was possible. For this purpose, an assumption was made by panelists. The broccoli heads were examined at the day of the PPA treatment. In a rough visual observation, changes were detected in color and texture for PPA-treated broccoli heads. Individual deviations are shown for the samples in Table 2. The texture of treated samples changed compared with untreated ones in a species-specific manner to being wilted after treatment. The color of the heads changed from lightly greyish and green to more greyish-green combined with grey buds. Another change in sensory aspects was noticed by the panelists for the odor of treated broccoli heads. It changed from fresh and species specific (untreated samples) to sweet, cooked, musty, and dry for treated samples. The panelists assumed that 5 of the 10 broccoli heads were treated,

TABLE 2: Results of sensorial experiments for untreated and treated broccoli heads

Number	Texture	Appearance	Odor	Assessment	PPA
1	Species specific	Green	Fresh, species specific	Untreated	No
2	Species specific	Green, greyish	Fresh, species specific, displeasing	Untreated	No
3	Species specific	Green	Fresh, species specific	Untreated	No
4	Species specific	Green	Fresh, species specific	Untreated	No
5	Species specific	Green, greyish	Fresh, species specific, dull	Untreated	No
6	Wilted	Greyish-green, grey buds	Sweet, cooked, stale	Treated	Yes
7	Wilted	Greyish-green, grey buds	Sweet, musty, dry	Treated	Yes
8	Wilted	Greyish-green	Sweet, musty, dry	Treated	Yes
9	Wilted	Greyish-green	Fresh, cooked, dry	Treated	Yes
10	Wilted	Greyish-green	Musty, dry	Treated	Yes

which was a correct assumption: 5 samples were untreated and 5 were treated. However, due to the fact that an investigation of this type is new (to our knowledge, one of the first worldwide), the examination conditions should be improved and more broccoli heads under different PPA treatment conditions and other environmental conditions (cooled environment) should be investigated in the future.

PPA treatment led to clearly detectable differences in texture, appearance, and odor for untreated and treated broccoli heads. These differences allowed an exact allocation of the samples by the panelists. The observed changes to the broccoli heads examined directly after PPA treatment were also found within the storage investigations. However, the microbiological results showed a strong PPA efficacy against *P. carotovorum* and *P. marginalis* already after a 5-min treatment combined with a 5 s plasma on time. A reduction in pretreatment time from 50 s (sensory and storage investigations) to 5 s (microbiological experiments) and a reduction of posttreatment time below 5 min will certainly lead to more acceptable results in sensory and storage examinations. Therefore, further investigations are needed.

C. Storage Results

Storage investigations were done for the direct influence of PPA treatment on the broccoli heads for cooled storage and shelf-life effects. PPA treatment of the broccoli heads had an influence on their color. The treatment led to visual detectable browning (Fig. 3). However, this effect was time dependent, meaning a 5-min PPA treatment resulted in negligible effects. Conversely, a 15-min treatment showed strong browning effects.

The scoring of PPA-treated broccoli heads during the storage under cooled conditions for 25 days (Fig. 4) showed losses in fresh mass that varied between 1.8% and

FIG. 3: Broccoli heads before and after PPA treatment. Reference (Ref) without PPA treatment is shown on the left and a 15-min PPA-treated broccoli head (P15) directly after treatment on the right.

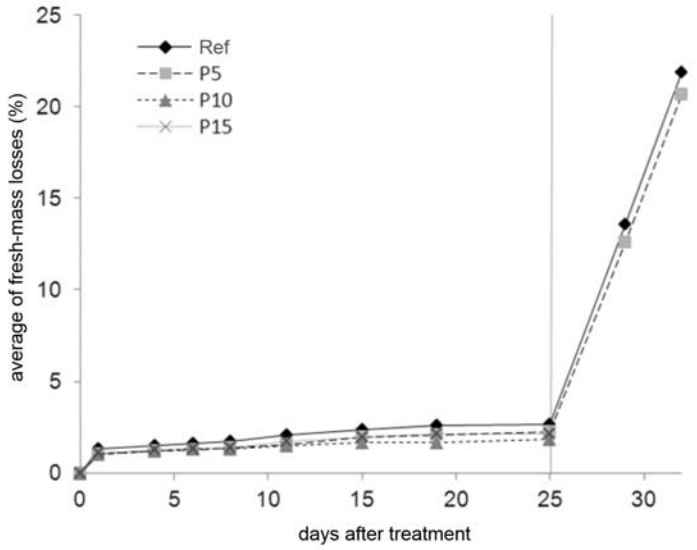

FIG. 4: Fresh mass losses (%) in dependence of storage time for PPA-treated broccoli heads and references. Initial weight was 625 g per head. Experiments were done with $n = 2$ for reference heads and 15-min treated samples and with $n = 3$ for 5-min and 10-min treated heads. Storage time was 25 days; shelf-life duration was an additional 7 days at 16°C.

2.6%. These are negligible compared with the losses seen during shelf-life examinations. After 32 days of storage, the average of fresh mass losses for the reference and the 5-min PPA-treated broccoli heads was 21%. Noticeable differences between different PPA treatments were not detected.

The described browning of broccoli heads after PPA treatment did not abate during cooled storage for 25 days. Changes in color were connected to changes in surface

structure (Fig. 5). The cuticle, typical in vegetables of the *Brassica* genus, was strongly affected by treatment. Another observed effect was the clearly reduced development of buds of PPA-treated heads compared with untreated ones during storage. An interesting observation was the color difference of PPA-treated broccoli heads inside single IFCO boxes. The lower layer was much greener than the upper one. One explanation might be the inhomogeneous filling of the treatment chamber by PPA. PPA consists of RNS, which are heavier than air. Therefore, it is possible that the concentration is increased closer to the bottom of the reaction chamber and thus at the bottom of the IFCO box.

FIG. 5: Macrophotography of broccoli head buds at the end of cooled storage, 25 days after PPA treatment. Reference (Ref) head is shown on the left and 15-min treated samples (P15) are shown on the right.

The increase of PPA treatment time showed the development of glassy-brown stem sections (Fig. 6) and brownish glazing (Fig. 7) during storage. The latter could be connected to damages of the stems' epidermis that is detectable only after storage.

The occurrence of head rot was low, so it was not possible to detect PPA's efficacy definitively against typical microbial load in this case.

FIG. 6: Glassy-brown stem cut from PPA-treated broccoli at the end of cooled storage, 25 days after treatment

FIG. 7: Brownish glazing of PPA-treated broccoli stems at different levels at the end of cooled storage, 25 days after treatment

The few heads with typical symptoms for head rot were seen for broccoli heads with 10 min of PPA treatment, especially for the reference heads. The occurrence of rot and *Botrytis* symptoms was negligible during the 25 days of storage at 1°C. In particular, *Botrytis* symptoms increased during shelf-life storage time at 16°C. At the end of 1 week, 3% of reference heads and 40% of 5-min PPA-treated heads showed *Botrytis*.

The fungi *Botrytis* is known as a parasite that infects weakened or senescent tissue. Therefore, the difference between untreated and treated heads may suggest damage to the plant's epidermal tissue and impairment of its barrier function against parasites by PPA treatment. This is consistent with the cuticle effects seen in Fig. 5.

At the end of shelf-life storage, the reference broccoli heads showed a clearly advanced senescence, whereas the 5-min PPA-treated heads had partial yellowing only (Fig. 8). In particular, exposed parts of the treated heads were green compared with ref-

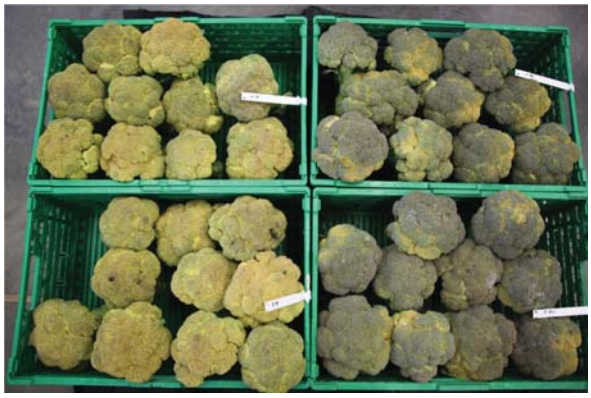

FIG. 8: Yellowing of broccoli heads at the end of 1 week of shelf-life storage at 16°C, 32 days after treatment. Reference (Ref) head is shown on the left and 5-min PPA-treated heads are shown on the right.

erence heads. The noticeable delayed decomposition of chlorophyll and delayed development of buds suggest an influence of PPA treatment on plant physiological processes in senescence.

IV. CONCLUSION

This study showed a possible and high inactivation of seven different microorganisms with microwave PPA. A significant dependency of inactivation efficiency due to microorganisms and their resistance to plasma–chemical components was detected. Preliminary investigations of sensory aspects of PPA-treated fresh broccoli heads showed influences on texture, appearance, and odor that may have been connected to PPA treatment. Comparable changes could be seen during storage and shelf-life investigations. Effects of PPA treatment on texture and appearance were also noticed. No influence on fresh mass weight and less yellowing during shelf-life storage was found for PPA-treated samples. Optimization of process parameters may increase the acceptance of sensory and storage effects.

The inactivation of microorganisms on fresh produce using nonthermal plasma depends on many parameters, such as plasma source, gas mixture, water content of gas and specimen, and the specimens' surface and microbiological species and concentration. However, our promising results and the advantages of PPA (low temperature, penetration of gaps, simple and cheap generation) offer a wide range of possible applications. Chemical interactions such as the function of RNS and reactive oxygen species with respect to microbial inactivation mechanisms and food interaction (food quality) should be further investigated.

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