Innovative Food Science and Emerging Technologies 22 (2014) 147–157

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Non-thermal atmospheric pressure plasma: Screening for gentle process conditions and antibacterial efficiency on perishable fresh produce

Matthias Baier [a], Mandy Görgen [a], Jörg Ehlbeck [b], Dietrich Knorr [c], Werner B. Herppich [a], Oliver Schlüter [a,*]

[a] Leibniz Institute for Agricultural Engineering, Max-Eyth-Allee 100, 14469 Potsdam-Bornim, Germany
[b] Leibniz Institute for Plasma Science and Technology, Felix-Hausdorff-Straße 2, 17489 Greifswald, Germany
[c] Department of Food Biotechnology and Food Process Engineering, Berlin University of Technology, Königin-Luise-Straße 22, 14195 Berlin, Germany

ARTICLE INFO

Article history:
Received 14 October 2013
Accepted 29 January 2014
Available online 8 February 2014

Editor Proof Receive Date 26 February 2014

Keywords:
Cold plasma
Chlorophyll fluorescence imaging
Physical sanitation treatment
Postharvest quality
Noninvasive methods
Disinfection

ABSTRACT

Fresh fruits and vegetables, destined to be eaten raw or minimally processed only, harbor the risk of conveying pathogenic microorganisms. Factors such as weather conditions, which favor survival or growth of microorganisms, and improper handling during cultivation or in the postharvest chain, can contribute to outbreaks of food-borne illness. Application of chemical sanitizers or physical treatments often shows a limited efficiency or does not meet consumer acceptance. Availability of gentle and effective techniques for disinfection of fresh produce, therefore, is highly desirable. Non-thermal gas plasma (NTP) treatment is a promising novel technique to reduce the microbial load on fresh fruits and vegetables. However, knowledge on practical applicability of NTP for fresh fruits and vegetables is very limited. In this study, chlorophyll fluorescence imaging (CFI) was used to elucidate suitable process parameters for application of an atmospheric pressure plasma-jet (kINPen 09, INP Greifswald, Germany) on corn salad, a perishable leafy green. Keeping a distance of 17 mm to the plasma-jet, corn salad leaves could be treated for up to 60 s at a fixed power (8 W) and 5 L min^{-1} of argon mixed with 0.1% oxygen. Surface temperature on leaves did never exceed 35.2 °C. Antibacterial tests were performed on corn salad, cucumber, apple, and tomato and achieved an inactivation of artificially inoculated *Escherichia coli* DSM 1116 of 4.1 ± 1.2, 4.7 ± 0.4, 4.7 ± 0, and 3.3 ± 0.9 log units, respectively, after 60 s treatment time. Additional tests with a dielectric barrier discharge plasma and indirect plasma treatment within a remote exposure reactor, fed by a microwave induced plasma torch, did not result in equivalent levels of quality retention as observed using the plasma-jet.

Industrial relevance: Development of gentle non-thermal disinfection methods aims to provide the industry with new tools to actively improve the microbial status of fresh produce beyond the preventive benefits of good hygiene practices and the limited efficacy of post-harvest washing. The presented study shows how cold plasma can be applied to heat-sensitive lettuce leaves without detrimental effects to product quality. The additional microbiological tests offer insights into the antibacterial capacity of cold plasma on different produce surfaces. The results contribute to prompt the development of appropriate large-scale plasma sources to establish a new plasma-based sanitation technique for fresh fruits and vegetables, which should also be implementable into running process lines.

1. Introduction

Fresh fruits and vegetables are subject to a more or less rapid degradation in postharvest. The need for fast distribution of perishable fresh produce from farm to fork limits possibilities of satisfactory and affordable microbiological analyses delivered in time for detection and, if necessary, discarding of contaminated batches. Hence, outbreaks of food-borne diseases, traced back to the consumption of fresh and minimally processed fruits and vegetables (CDC report, 2012; RKI report, 2011), remain a serious issue. Good Agricultural Practices (GAP), implementation and compliance of standards and certificates are basic steps in terms of food safety. Sporadic presence of pathogens on fresh produce, however, requires effective sanitation techniques to reduce microbial loads without any negative effects on product quality.

During recent years, quite a number of studies dealt with chemical sanitizers or physical treatments (Ramos, Miller, Brandão, Teixeira, & Silva, 2013) such as high hydrostatic pressure (Schlüter, Foerster, Geyer, Knorr, & Herppich, 2009) and UV- or gamma-irradiation (Hassenberg et al., 2012; Lescano et al., 1993; Poubol et al., 2010; Sothornvit and Kiatchanapaibul, 2009). However, the abovementioned methods are either not fully harmless for consumers or could not achieve the desired success, or even negatively affected the produce quality. Therefore, alternative sanitizers, which

* Corresponding author. Tel.: +49 331 5699 613.
E-mail address: oschlueter@atb-potsdam.de (O. Schlüter).

1466-8564/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ifset.2014.01.011

guarantee a low microbial load in combination with retaining high produce quality during shelf-life are of great interest.

A promising physical approach is the application of non-thermal plasma (NTP) generated at atmospheric pressure. In the physical context, the terms "non-thermal" or "cold" plasma are not referred to the actual temperature. They rather reflect the absence of a thermodynamic equilibrium between highly energized electrons and the far less affected main part of gas atoms and molecules, which can, in turn, result in gas temperatures near ambient (Schlüter et al., 2013). A process gas can be transformed into NTP by a strong electric field. Induced excitation and partial ionization of the process gas molecules lead to the concomitant formation of various reactive chemical species, such as ions and radicals, heat, and UV light, which all together potentially react with the microbes adhesive to the food surfaces (Keener, 2008). The actual role of the respective single microbicidal active agents, from just detectable presence up to a predominant part in inactivation, strongly depends on the plasma source used, the process parameters employed, and the process gas used. Different principles of plasma generation and a broad range of geometrical arrangements offer both advantages and limitations for each type of plasma source for possible fields of application and result in high variation of antimicrobial efficiency (Ehlbeck et al., 2011). Additionally, one and the same plasma source may offer significantly different modes of application. A plasma-jet for instance, can be applied to a sample directly, if the treatment distance is held short enough that the plasma filaments are touching the sample surface. This mode allows for interactions of the complete composition of plasma species with the sample surface. If there is any space between glowing discharge and sample surface, the treatment becomes semi-direct, avoiding the interaction with charged particles. If the distance is further increased, the type of treatment becomes indirect and only long-lived plasma species can reach the sample. Thus, these variations are reflected by the still small amount of available data on the disinfection of fresh produce using non-thermal plasma (Schlüter et al., 2013).

Critzer, Kelly-Winterberg, South, and Golden (2007) used a one atmosphere uniform glow discharge plasma (OUAGDP) to reduce strains of *Escherichia coli* (*E. coli*) O157:H7, *Salmonella* sp. and *Listeria monocytogenes*, artificially inoculated on apples, cantaloupe, and iceberg lettuce by at least 2 log units within a few minutes. With an indirectly applied gliding arc plasma, Niemira and Sites (2008) inactivated *E. coli* O157:H7 and *Salmonella* Stanley on apple surfaces by 3 log units at temperatures between 40 °C and 51 °C and 3 min treatment time. A plasma-jet was applied on the pericarps of mangos and melons yielding 3 log unit reductions of *E. coli* and different spoilage microorganisms (*Saccharomyces cerevisiae*, *Pantoea agglomerans*, and *Gluconobacter liquefaciens*). In their study, Perni, Liu, Shama, and Kong (2008) recorded a temperature rise of 5 °C at 1 cm distance from the plasma-jet nozzle. Further investigations on cut fruit surfaces, however, showed a resistance of microorganisms due to migration into the fruit tissue (Perni, Shama, & Kong, 2008). In a recent study, Fernández, Noriega, and Thompson (2013) used a commercially available nitrogen plasma-jet to inactivate *Salmonella* Typhimurium on fresh produce. Showing pronounced tailing effects, bacteria were reduced by 2.72, 1.76, and 0.94 log units on lettuce, strawberry, and potato, respectively after 15 min. Similar results were obtained after indirect treatment of romaine lettuce and cocktail tomatoes in the afterglow of a needle array at high voltage, leading to a 1.6 log unit reduction after 10 min (Bermúdez-Aguirre, Wemlinger, Pedrow, Barbosa-Cánovas, & Garcia-Perez, 2013). Direct treatment for up to 30 s using an atmospheric pressure plasma-jet inactivated *E. coli* on corn salad by 2.1 up to 3.6 log units at 10^7 and 10^4 cfu cm^{-2} initial bacterial loads, respectively (Baier et al., 2013).

Disinfection of the physiologically active fresh produce is a challenging task, since prevention of detrimental effects on the metabolism of products is all-dominant to maintain the naturally limited valuable time in the postharvest chain. Data on quality aspects of produce after plasma treatment is still scarce and mainly limited to measurements of the outer appearance. Vleugels et al. (2005) reported minor discoloration of bell peppers due to plasma treatment compared to high color variations between untreated samples. Only insignificant color differences ($p > 0.05$) were measured on romaine lettuce and cocktail tomatoes (Bermúdez-Aguirre et al., 2013) and very small color differences were detected using a plasma-jet setup on cut surfaces of cucumber, carrot and pear slices (Wang et al., 2012). In the downstream of an array of dielectric barrier discharges (DBDs), Tappi et al. (2013) observed a reduced enzymatic browning and a tendentially retarded overall metabolic activity in fresh-cut apple slices, as measured for up to 24 h after treatment. In a recent study (Misra et al., 2014), further information on quality aspects was obtained after indirect in-package treatment of strawberries using a DBD. Respiration or changes of color and firmness did not change significantly within 24 h, while indigenous microflora was reduced by 2 log units at the same time. However, still little is known about the potential impact of plasma on plant metabolism and following effects during extended storage of more than 24 h. In a first study (Baier et al., 2013), light was shed on the basic feasibility of non-thermal plasma applied directly on fresh corn salad leaves. Treatment by an experimental prototype of an atmospheric pressure plasma-jet at moderate power led to at least temporary decline of photosynthetic efficiency. This treatment was accompanied by serious erosion phenomena on the upper epidermis (Grzegorzewski, Ehlbeck, Schlüter, Kroh, & Rohn, 2011). Since analysis ended 24 h after treatment, the question of potential delayed effects of plasma on the physiology of leaves has not been addressed, yet.

Therefore, using an advanced atmospheric pressure plasma-jet, the aim of this study was to screen for gentle treatment conditions excluding any immediate or delayed effects on lettuce leaves until the end of a three-day storage period. Non-destructive chlorophyll fluorescence imaging was applied to measure the impact of direct plasma contact compared to an indirect treatment mode. Process settings, suitable for thin lettuce leaves, were tested for their antibacterial efficiency on corn salad leaves and voluminous fresh produce.

A further aspect that has not been covered yet is a comparative exploration of alternative plasma sources. Uniform exposure of whole pieces of fruit and vegetables and higher numbers of samples are essential steps for further development of plasma techniques for both research of plasma effects on food systems, and to fathom possibilities for process engineering. A common technique used in many studies, which enables plasma applications on areas of several square centimeters, is the generation of plasma between the plate electrodes of a dielectric barrier discharge (DBD). DBDs offer desirable sporicidal efficiencies at short-term treatment of less than 1 min (Heise, Franken, Neff, Wunderlich, & Muranyi, 2004). Another aspect is the reduction of expectable high operating costs if an expensive noble gas such as argon is used. Using ambient air as process gas instead, exhibits a substantial improvement for the development of large-scale plasmas at ambient pressure. Experiments with air plasma, allowing indirect treatment of larger workpieces such as medical instruments within a remote exposure reactor were reported by Gadri et al. (2000). Additionally, separation of plasma generation zone and site of treatment can offer new options for plasma sources. Sufficient distance between discharge and sample enables means to cool detrimental heating of the plasma gas. Consequently, even a thermal plasma source such as a plasma torch could be used, if its exhaust is cooled on its way to the treatment site, resulting in non-thermal conditions (Ehlbeck et al., 2011; Schnabel et al., 2012).

In the present study, a surface DBD and a recently developed exposure chamber fed by a microwave driven plasma torch (PLexc®, INP, Greifswald, Germany) were used for treatment of corn salad leaves. Treatment durations were adapted to those of the plasma-jet treatment of the main part of this study. The impact of the alternative plasma sources was examined using chlorophyll fluorescence imaging.