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Impact of postharvest UV-C and ozone treatments on microbiological properties of white asparagus (*Asparagus officinalis* L.)

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Summary

To meet the increasing demand for safe and high quality fresh white asparagus and the recent food safety regulations, optimization of postharvest handling, processing and storage is essential. Modern sanitation techniques relying on physical methods and/or Generally Recognized As Safe (GRAS) compounds are desired for reducing microbiological spoilage. To evaluate the effects of aqueous ozone and UV-C on the microbial load of spears, samples were UV-C irradiated (254 nm, 1 kJ m^{-2}) and/or washed with ozonated water (approx. 3 ppm or 4.5 ppm at 10 °C), and analyzed at three times during a four day storage. Also, the potential effects of initial natural microbial loads, and precondition of the spears in terms of water and sugar contents on the responsiveness of asparagus to these treatments were determined in detail over four growing seasons. The initial microbial loads (mould and yeasts, and aerobic mesophilic total bacterial counts) of white asparagus spears varied considerably during the different harvest seasons of this four-year study. This variability could not be explained by the variance of climatic conditions nor by the respective water and sugar content. Furthermore, there was never a clear cut relation of the initial microbial load and the growth of pathogens during four-day storage at 20 °C in nearly water vapour saturated atmosphere. Neither washing the spears with ozonated water (3 or 4.5 ppm) nor treating them with UV-C radiation (1 kJ m^{-2}) systematically and significantly affected their microbial loads during storage. In addition, the assumption that a combination of both treatments could synergistically improve the effect of each treatment could not be verified during this long-term study. In conclusion, microbial load and pathogen development in asparagus spears are highly persistent and, thus, to meet hygienic requirements further investigations will be necessary.

Introduction

White asparagus (*Asparagus officinalis* L.) is a very important and popular crop in Germany. In 2010, it was cultivated on an area of 22.900 ha, representing 20 % of the total area used for vegetable production (Statistisches Bundesamt, 2011). Asparagus is considered as a nutritionally highly valuable vegetable (Makus, 1994; Siomos, 2003), containing important antioxidant and anticancerogenic compounds (Eichholz et al., 2012). At present, asparagus is mainly purchased as fresh commodity; the demand for minimally processed fresh-cut convenience product, however, largely increased during recent years. As a consequence, quality assurance has to now focus both on the retardation of metabolic processes, e.g. reduction of undesired spear toughening or losses of value adding components (Huyskens-Keil and Herppich, 2012; Huyskens-Keil et al., 2011), and on meeting the recently intensified hygienic requirements. Total economic losses within the entire postharvest chain have been estimated as high as approx. 45 % for fresh fruits and vegetables in Europe (Gustavsson et al., 2011). For asparagus, supermarket losses alone may range between 8 and 11 % in the USA (Buzby et al., 2009); this is, to a large part, due to microbiological spoilage (Barth et al., 2009; Kadau, 2005). Consequently, for asparagus, optimization of postharvest handling and processing as well as storage is essential to reduce economic losses and improve food supply chain management.

Former standard chemical sanitation treatments applying chlorine or methylbromide include the risk of hazardous by-products formation, such as carcinogenic trihalomethane (THM) (Brungs, 1973; Wei et al., 1985). As a conseuqence, these applications are meanwhile forbidden by German law (LFBG, 2011). More recently, newly developed hygienisation techniques progressively include physical treatments (e.g. UV-irradiation, gamma-irradiation) or fumigation with Generally Recognized As Safe (GRAS) compounds such as ozone (Jamieson et al., 2009). Indeed, UV-irradiation and ozone fumigation or washing with ozonated water gain more and more importance in this context. Numerous studies have already been focused on the bactericidal and fungicidal capacity of ozone and UV-C treatments (Karaca and Velioglu, 2007; Hassenberg et al., 2007 and 2008; Escalona et al., 2010; González-Aguilar et al., 2010; Horvitz and Cantalejo, 2012).

As a strong oxidiser, ozone exhibits efficient bactericidal and fungicidal properties and very effectively destroys various microorganisms by oxidative breakdown of carbon residues in the washing water or adhering to the surface of the produces. Ozone is efficent at low concentrations, it requires only a short contact times, does not generate hazardous residues in or on the treated product and rapidly auto-decomposes to oxygen (Jamieson et al., 2009). Applied as gas or dissolved in water, ozone has been used to sanitizing produces, thus, extending storage time of perishable food (Hassenberg et al., 2008). In addition, as a component of the water desinfection system, ozonation can also be implemented into excisting washing processes (e.g. Artés et al., 2009). Hence, gaseous and solved ozone has been successfully applied to several produces such as tomatoes, strawberries, grapes and plums (Tzortzakis et al., 2007; Rodoni et al., 2010), carrots (Hassenberg et al., 2008), apples (Achen and Yousef, 2001), lettuce (Hassenberg et al., 2007) and cantaloupe (Rodgers et al., 2004).

On the other hand, UV irradiation is predominantly applied for effective surface decontamination. UV-C (wavelength range 190 nm to 280 nm) directly damages microbial DNA (Artés et al., 2009) or induces the biosynthesis of plant secondary metabolites (Schreiner et al., 2012) which, in turn, may inhibit or retard germination of bacterial or fungal spores (Perkins-Veazie et al., 2008). As ozone, UV-C irradiation does not produce harmfuls chemical residues but is lethal to most types of microorganisms. In addition, it does not require extensive safety equipment during utilization (Artés et al., 2009).

UV-C irradiation effectively retarded microbial growth on sliced zucchini squashes even at moderately cold temperatures of 5 °C and 10 °C during 12 days of storage (Erkan et al., 2001). UV-C irradiation also effectively reduced *Salmonella* spp. and *Escherichia coli* O157:H7 on 'Red Delicious' apples, leaf lettuce and tomatoes (Yaun et al., 2004). Further, UV-C treatment controlled *Botrytis cinerea* and *Penicillium expansum* on 'Empire' apples (Wilson

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et al., 1997), showed germicidal effect on total viable count on fresh-cut apples (MANZOCCO et al., 2011) and significantly inhibited *Monilinia fructicola* on 'Yali' pears (LI et al., 2010). For green asparagus, the effect of UV-C irradiation on the quality were tested (POUBOL et al., 2010); information about its influence on the natural microflora of white asparagus spears, however, are not available.

Hence, one aim of the presented investigation was to evaluate the effect of ozonated water and UV-C irradiation, or a combination of both, on the microbial load of white asparagus spears during shelf-life. For this purpose, spears were treated with UV-C and/or ozonated washing water and were analyzed at three times during four day storage. Untreated spears were used as controls. In addition, it has not yet been determined in detail whether the initial natural microbial load may potentially influence the responsiveness of white asparagus spears to UV-C and/or ozone treatments. This also applies to important spear quality parameter such as water and sugar contents. These attributes indicate the physiological status of the produce, which is of importance for the growth of bacteria and for the germination of fungal spores (COATES and JOHNSON, 1997; SHOLBERG and CONWAY, 2000). Consequently, all the above criteria were tested over the range of four growing seasons and analysed in relation to the effectivness of UV-C and/or ozone treatments.

Material and methods

Plant material and experimental setup

In four independent experiments (2006, 2008, 2009 and 2011), white asparagus spears ('Gijnlim'), freshly harvested from commercial fields near Berlin (2006 and 2008: Spargelgut Diedersdorf, Diedersdorf, Germany; 2009 and 2011: Spargelhof Nottebohm GbR, Kartzow, Germany), were immediately transported to the laboratory, washed, sorted (according EC quality standard class I), cut to a length of 22 cm (mean spear diameter: 1.8 ± 0.2 cm) and randomly separated into 12 batches of approximately 500 g.

Thereafter, three batches of spears each were either kept a) untreated (controls), b) submerged in ozonated water (approx. 3 ppm (2006, 2008 and 2011) or 4.5 ppm (2009) at 10 °C (thermostat 45; Haake, Karlsruhe, Germay) for 30 s, c) irradiated with UV-C (254 nm; VL-6C, 6 W-254 nm Tube, Power: 12 W, Vilber Lourmat, Marne-la-Vallee, France) at 1 kJ m^{-2}, or d) both washed with ozonated water and UV-C-irradiated. Ozone was generated at rates of 0.02 g min^{-1} by a 'Bewazon 1' ozone generator (BWT Water Technology Ltd., Schriesheim, Germany); ozone concentrations were measured with a DR 2800 photometer (Hach Lange GmbH, Berlin, Germany) applying a complementary chlorine/ozone cuvette test (Dr. Bruno Lange GmbH & Co., Düsseldorf, Germany). In addition, to evaluate the effect of tap water washing on microbial flora (e), spears were submerged in tap water at 10 °C for 30 s in 2011. After treatments, spears were stored at 20 °C in water vapour saturated atmosphere for up to 4 d.

All climatic data given were continuously recorded (automatic weather station, TOSS GmbH, Potsdam, Germany) at the Leibniz-Institute for Agricultural Engineering Potsdam-Bornim, Potsdam, Germany.

Microbiological analysis

For the determination of the aerobic mesophilic total bacterial counts (2009 - 2011), yeast and mould counts (2006 - 2011), 3 asparagus spears each were used for the control and each treatment. Therefore, 5 g from the apical, the middle and the base section of each spear was placed in a stomacher bag under sterile conditions. After adding 45 ml of Ringer solution, the resulting mixture was homogenized in a stomacher (Bagmixer 400, Interscience, St. Nom, France) for 2 min and aliquot diluted. For the analysis of the aerobic mesophilic total bacterial count, 100 µl of it was spread plated on Plate Count Agar (PCA, plates, Merck, Darmstadt, Germany) and incubated at 30 °C for 2 d. In the case of yeast and mould counts, 100 µl was plated on Rose-Bengal Chloramphenicol Agar (RBC plates, Merck, Darmstadt, Germany) and incubated at 25 °C for 7 d. Determination were performed twice for each spear.

Analysis of quality parameter

On days 0 (n = 12), 2 and 4 (n = 6) of the experiment, spears of each treatment were randomly taken out of storage. For each spear, fresh mass (*FM*, electronic balance BP 210 S, Sartorius AG, Göttingen, Germany) and total length were determined. At positions 2.5 cm, 7.5 cm, 12.5 cm and 18 cm from the spear base, the diameters of the spears were determined by means of an electronic calliper. At these positions, the spears were cut, and from each section, tissue sap was extracted by freezing/thawing procedure. This sap was analysed for TSS (total soluble solid content; PR 1 digital refractometer, Leo Kuebler GmbH, Karlsruhe, Germany), osmotic content (VAPRO 5520, Wescor Inc., Logan, UT, USA) and sugar content (HPLC). All sections were dried in an oven (85 °C) to constant mass for determination of the spear dry mass (DM). From fresh and dry mass, spear water content was calculated as WC_{DM} = (FM-DM)/DM and values were given in g g_{DM}^{-1}. The osmotic content was related to spear dry mass and given in mol kg_{DM}^{-1}.

For HPLC analysis of free sugar content, 0.15 ml of tissue sap were diluted with distilled water to a volume of 1.5 ml and cleared by micromembrane filtration (0.45 µm PVDF-Spritzenfilter, Rotilabo®, Carl Roth GmbH + Co. KG, Karlsruhe, Germany). After clearing, the extracts were stored on ice until HPLC-analysis with a Dionex HPLC (Dionex, Sunnyvale, USA) using a Eurokat H column (300 mm x 8 mm, 10 µm) and 0.01 N H_2SO_4 as the mobile phase (rate: 0.8 ml min^{-1} at 63 Pa) and a refractive index detector (RI 71, Shodex, Techlab, Erkerode, Germany).

Statistical analysis

All data were statistically analysed (ANOVA) with WinSTAT (R. Fitch Software, Staufen, Germany). Treatments means were statistically compared using the Duncan's multiple range test ($P < 0.05$).

Results

Initial natural microbial loads of freshly harvested asparagus spears and climate data

In general, the loads of freshly harvested spear with yeasts and moulds were low, often below detection limits, and ranged up to $1.1 \cdot 10^5 \pm 2.9 \cdot 10^4$ cfu g^{-1} and $4.6 \cdot 10^3 \pm 2.6 \cdot 10^3$ cfu g^{-1}, respectively (Tab. 1). No significant effects of harvest season or year on these

Tab. 1: Initial natural microbial loads (yeasts, moulds and total microbial counts) of freshly harvested 'Gijnlim' asparagus spears, as determined during four harvest seasons.

Sample year	yeasts (cfu g_{FM}^{-1})	moulds (cfu g_{FM}^{-1})	total bacterial counts (cfu g_{FM}^{-1})
2006	$1.1 \cdot 10^5 \pm 2.9 \cdot 10^4$	$2.6 \cdot 10^3 \pm 1.1 \cdot 10^3$	nm
2008	$1.7 \cdot 10^4 \pm 2.6 \cdot 10^4$	$4.0 \cdot 10^3 \pm 2.6 \cdot 10^3$	nm
2009	$< 10^3 \pm 0$	$2.5 \cdot 10^3 \pm 2.6 \cdot 10^3$	$1.7 \cdot 10^5 \pm 5.8 \cdot 10^4$
2011	$< 10^3 \pm 0$	$< 10^3 \pm 0$	$6.0 \cdot 10^5 \pm 9.6 \cdot 10^5$

nm – not measured

parameters could be detected. This was valid for both field locations. It also applied for the total microbial counts, which were found to be $4.1 \cdot 10^5 \pm 2.2 \cdot 10^5$ cfu g^{-1} on average.

There was a broad range of weather conditions during the three months of asparagus harvest seasons among all years investigated (Tab. 2). In 2011, climate was warm and sunny, with a constant but moderate precipitation. In contrast, in 2008, it was rather cold during harvest season with few rain events in May. On the other hand, 2009 was warm and wet compared to the other years.

In nearly 85 % of all samples, irrespective of any treatment or harvest year, *Penicillium* spec. moulds could be detected (Tab. 3). In addition, *Fusarium* spp., *Cladosporium* spec. and *Acremonium* spec. showed a relatively high frequency. On the other hand, moulds of *Alternaria* spec., *Botrytis cinerea*, *Paecilomyces* spec. were only rarely isolated.

Tab. 3: Frequency of detected mould species on asparagus spears independent of treatment and harvest year.

Species	Frequency (%)
Acremonium sp.	36.8
Alternaria sp.	5.3
Aspergillus sp.	15.8
Botrytis cinerea	5.3
Cladosporium sp.	36.8
Fusarium sp.	68.4
Paecilomyces sp.	5.3
Penicillium sp.	84.2

Ozone and UV-C treatment effects on microbial loads of stored asparagus spears

Analysed directly after application, no effect of any postharvest treatment on yeast and mould counts, and aerobic mesophilic total bacterial counts could be detected; hence, data were not shown. During storage, both counts of yeasts and moulds tended to increase by more than one log in all experiments, irrespective of the treatments (Fig. 1). In most cases, however, changes were statistically not significant. Nevertheless, after four d of storage, a slight inhibition of yeast growth was always observed in treated spears compared to controls, except in 2011 (Fig. 1 A, C, E, G). In addition, no clear cut systematic effects of any treatment were monitored although the combined application of both O_3 and UV tended to yield the lowest yeast loads of spears at the end of storage. Results of mould counts were more inhomogeneous (Fig.1). Only in the first experiment, all treatments significantly inhibited mould growth after four day storage compared to controls (Fig. 1 B). In contrast, treatments even yielded partially significantly higher mould counts in the other experiments (Fig. 1 D, F, H).

Also, none of the treatments tested could significantly inhibit the increase of the aerobic mesophilic total bacterial counts during four days of storage (Fig. 2). In addition, washing the spears with ozonated water at a higher concentration of 4.5 ppm (Fig. 2 A) compared to that of 3 ppm (Fig. 2 B) did not result in lower aerobic mesophilic total bacterial counts. Furthermore, combination of ozonated washing water and UV-C irradiation could not significantly reduce to increase of total bacterial counts (Fig. 2).

Analysis of quality parameters

Initial produce quality largely and significantly varied between the different harvest seasons as indicated by spear water content, osmotic content, TSS and total sugar contents (Fig. 3). Spear quality was, in general, low in 2006 (lowest water content, osmotic and total sugar content, and low soluble solid); while it was highest in 2010 (moderate water content, high osmotic, soluble solid and total sugar content). In 2009, spears had the highest mean water content but, on the other hand, only moderate osmotic, soluble solid and total sugar contents.

In all years, mean water content of controls did not change during four days of storage (Fig. 3; cont-4). In contrast, all other parameters (osmotic content, TSS, sugar content) declined in untreated spears during storage. These differences were statistically significant in 2006 and 2008, but not in 2009 and 2011. Compared to the controls, quality parameters were not systematically affected by either treatment. Nevertheless, spears exposed to the combined treatment

Tab. 2: Daily means (± SD) of air temperature, relative air humidity, global radiation and total monthly precipitation during time of asparagus production in all years of investigation.

Sample year	Month	mean daily air temperature (°C)	mean daily rel. humidity (%)	mean daytime global radiation (W m^{-2})	total monthly precipitation (ml $month^{-1}$)
2006	April	8.9 ± 3.4	78.9 ± 11.0	142 ± 58	50.4
	May	14.8 ± 2.6	66.8 ± 14.4	212 ± 63	36.4
	June	19.0 ± 4.5	67.5 ± 10.3	258 ± 64	13.3
2008	April	8.6 ± 3.5	79.4 ± 12.8	148 ± 83	64.2
	May	12.2 ± 4.5	63.4 ± 9.6	251 ± 54	6.3
	June	11.0 ± 3.7	60.4 ± 11.8	267 ± 68	36.3
2009	April	13.2 ± 2.8	61.4 ± 10.3	267 ± 68	2.1
	May	15.3 ± 3.0	69.5 ± 11.1	213 ± 78	73.9
	June	16.4 ± 3.1	74.8 ± 7.7	201 ± 71	62.8
2011	April	13.4 ± 3.3	70.1 ± 11.9	170 ± 65	29.0
	May	16.2 ± 4.4	64.7 ± 10.0	237 ± 61	20.6
	June	19.4 ± 3.1	70.6 ± 9.8	244 ± 79	25.0

showed significantly higher retention of total sugars and osmotic content in 2011 (Fig. 3).

Discussion

COATES and JOHNSON (1997) stated that a wide range of preharvest factors may affect the postharvest susceptibility of produces to phytopathogens. Among others, weather is assumed to have a prevailing effect (WEBB and MUNDT, 1978; SHOLBERG and CONWAY, 2000). Although climatic conditions during the different harvest seasons were highly variable, ranging from dry, sunny and hot in 2011 and wet, cloudy and cool in 2010 to, wet, sunny and warm in 2009 neither yeast or mould counts nor total bacterial counts of asparagus spears reflected this variability in the present study. From the climatic conditions, highest microbial load could be expected in 2009 (SHOLBERG and CONWAY, 2000); however, in this year all counts were relatively low. On the other hand, initial counts were highest in 2006 with its moderate climate with relatively low precipitation. In this context, GOßMANN et al. (2008) could not verify a clear influence of temperature and, especially, precipitation on the microbial loads of asparagus spears from different locations with *Fusarium* species.

The fact that overall climatic conditions seem to have a low effect on the initial microbial counts may at least partially depend on the sandy soils in which the analyzed asparagus were cultivated in Brandenburg region. Though the area is less suitably for the cultivation of asparagus (MARTINEZ et al., 2007), these soils are mostly well-drained, thus preventing water logging, which would facilitate the growth of fungal pathogens (BRÜCKNER et al., 2008). Hence, only low loads of soilborne moulds were found in this region (BRÜCKNER et al., 2008).

On the other hand, the generally low microbial loads found during all years of investigation agreed well with early finding of WEB and MUNDT (1978). These authors reported negligible contaminations on

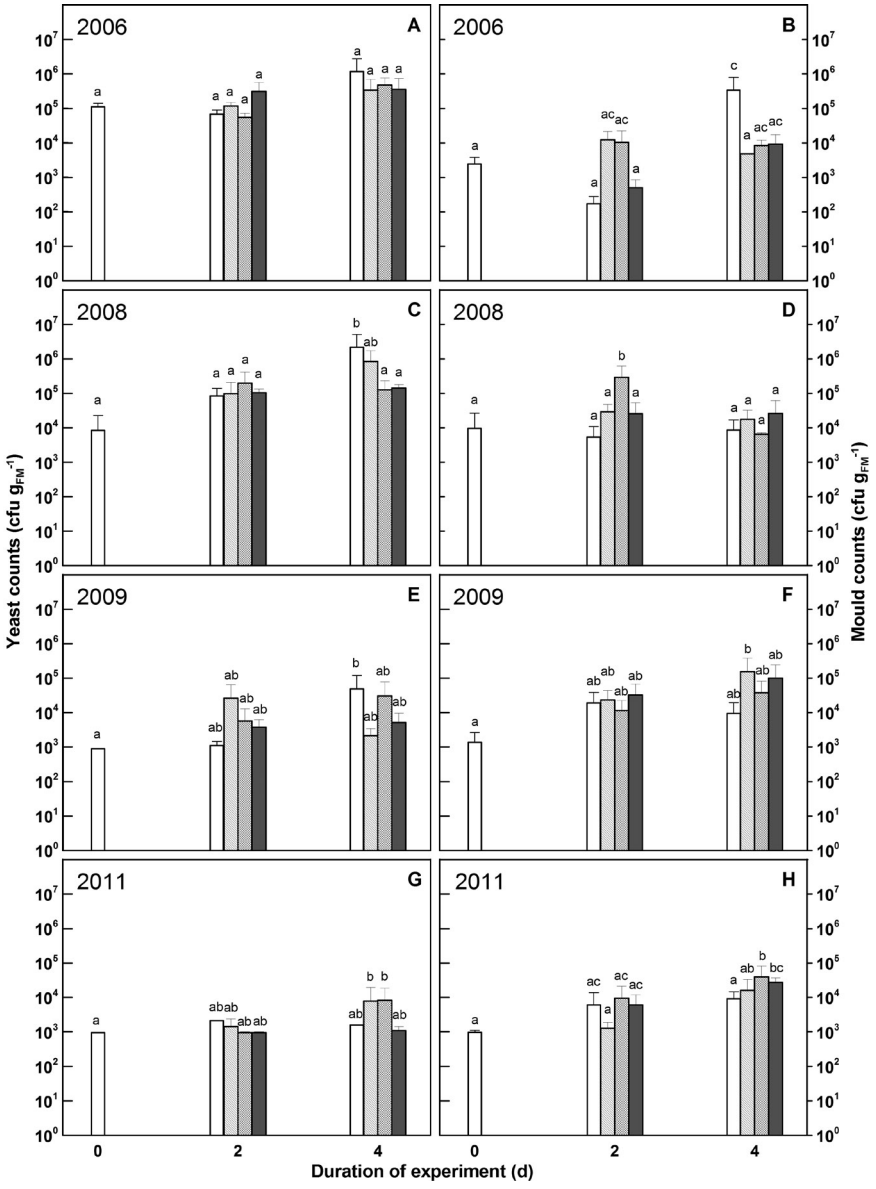

Fig. 1: Means (± SD; n = 3) of yeast counts (A, C, E, G) and mould counts (B, D, F, H) of fresh and stored (at 20 °C in water vapour saturated air for up to 4 days) asparagus spears in 2006, 2008, 2009 and 2011. Before storage, spears were either untreated (□ open bars), washed with ozonated water (90 s, 3 or 4.5 ppm; ▨ hatched bars), irradiated with UV-C (1 kJ m^{-2}; ▩ crossed bars) or both washed with ozonated water and irradiated (■ filled bars). Different letters indicate significant differences between means (Duncan's multiple range test, $P < 0.05$).

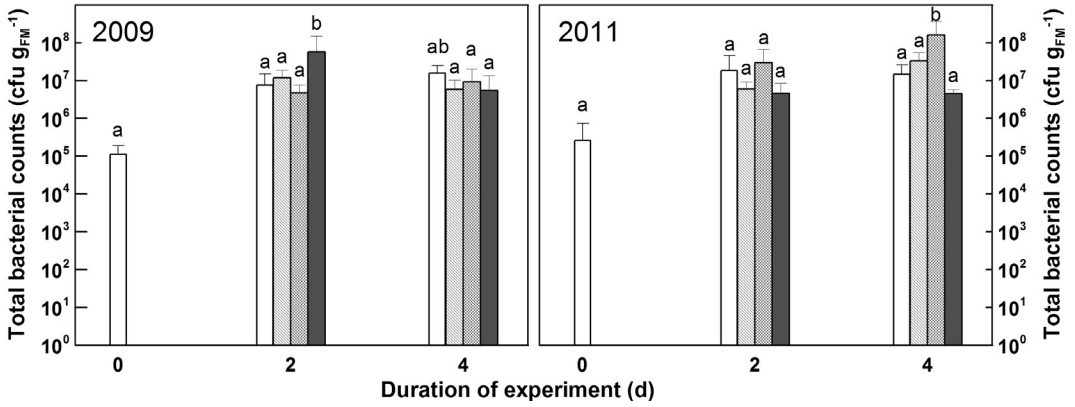

Fig. 2: Means (± SD; n = 3) of total bacterial counts of fresh and stored (at 20 °C in water vapour saturated air for up to 4 days) asparagus spears in 2009 and 2011. Before storage, spears were either untreated (open bars), washed with ozonated water (90 s, 3 ppm (2009) or 4.5 ppm (2011); hatched bars), irradiated with UV-C (1 kJ m^{-2}; crossed bars) or both washed with ozonated water and irradiated (filled bars). Different letters indicate significant differences between means (Duncan's multiple range test, $P < 0.05$).

green asparagus spears compared to various other vegetables such as beans or cucumbers. In the present study, this finding applies both for aerobic mesophilic total bacterial as well as mould and yeast counts. The initial loads of the former closely reflect those reported by Berrang et al. (1990; 10^4 - 10^5 cfu g^{-1}) and Lescano et al. (1993; $5.0 \cdot 10^4$ cfu g^{-1}). In contrast, starting counts of fungal pathogens were more than one log lower than reported earlier for white 'Argenteuil' spears from Argentina (Lescano et al. 1993; $7.2 \cdot 10^4$ cfu g^{-1}).

The finding that most of the fungal pathogens identified in the present study, belong to *Penicillium* spec., *Fusarium* spec., and *Cladosporium* spec., while moulds of *Aspergillus* spec., *Alternaria* spec. and *Botrytis cinerea* contributed to only a minor and highly variable degree to the overall counts, closely reflects earlier reports on the endophytic fungal loads of asparagus spears (Web and Mundt, 1978; Kadau, 2005). On the other hand, moulds of *Acremonium* spec. and *Paecilomyces* spec. have not been described for asparagus before. Especially the former moulds, however, seem to be a regular microbial load in Brandenburg asparagus spears. In contrast to Goßmann et al. (2008), the occurrence and prevalence of *Fusarium* spec. did obviously not depend on the sampling location or sampling date.

Although the initial microbial load varied considerably during the different harvest seasons, there was never a clear cut relation to the growth of pathogens during storage. Although spears stored under conditions favourable for microbial growth (Tamm and Flückiger, 1993; Samson et al., 2010), i.e. 20 °C and water vapour saturated atmosphere, their growth was generally rather limited and changes highly variable, not at least in the 2011 season.

Huyskens-Keil et al. (2012) reported a significant effect of post-harvest applied aqueous ozone and UV-C on textural properties of white asparagus spears. In the present study, it has been hypothesized that the initial natural microbial load might potentially influence the responsiveness of white asparagus spears to UV-C and/or ozone treatments. The higher the initial load the more effective might the phytopathgens spread (Sholberg and Conway, 2000) and, as a consequence, the less effective could the treatment be. In addition, high produce water content and, on the other hand, sugar content might provide an optimal growing medium for pathogenes. Up to now, this has not yet been studied in detail. However, from the presented results it seems clear now that these factors did not affect the microbial growth in control samples nor did they influence the effects of any of the chosen treatments.

In fact, none of the investigated treatments yielded a satisfying reduction or control of the microbial load on fresh, unprocessed white asparagus spears during storage for up to four days. This is surprising because both ozone (Achen and Yousef, 2001; Tzortzakis et al., 2007; Hassenberg et al., 2007; 2008; Horvitz and Cantalejo, 2012) and UV-C radiation (Wilson et al., 1997; Marquenie et al., 2002; Allende et al., 2006; Li et al., 2010; Escalona et al., 2010) have been proven to be effective for this purpose in many fresh produces (Karaca and Velioglu, 2007). On the other hand, Poubol et al. (2010) also reported that even UV-C irradiation of 3.6 kJ m^{-2} did not show any inhibitory effect on the growth of the natural microflora including total microbial counts, coliforms and fungi in green asparagus. Maybe the effect of radiation may increase with radiative doses as shown for gamma irradiation (Lescano et al., 1993). However, further enhancement of UV doses may be limited by the potential sensitivity of produces to this radiation.

Very similar unsatisfying ambiguous results were obtained for washing with ozonated water. This is in contrast to the findings of Sothornvit and Kiatchanapaibul (2009), who reported a reduction of microorganisms by nearly 1 log after washing with ozonated water (0.1 mg L^{-1}; 10 °C, 15 min) compared to tap water-washed spears. In addition, Horvitz and Cantalejo (2012) obtained a successful washing time dependent decline in microbial populations when washing bell pepper with ozonated water (1 ppm). In this experiment, however, gaseous ozone (0.7 ppm) proved to be more effective. In addition, chlorine solution seemed to be advantageous over ozone (Sothornvit and Kiatchanapaibul, 2009; Horvitz and Cantalejo, 2012). On the other hand, the use of latter solution is limited by new legislations (Hassenberg et al., 2008; LFBG, 2011).

The application of the higher ozone concentration of 4.5 ppm did not result in a better inhibition of microorganisms compared to the application of 3 ppm. Further increase in ozone concentration is limited by the solubility of this gas, which strongly depends on the temperature and decreased with increasing water temperature (Langlais et al., 1991). In this context, Achen and Yousef (2001) proposed the use of continuous bubbling of ozone gas in the washing water to guarantee a high ozone concentration. Nevertheless, it has been shown that high concentration ozone treatment may potentially result in produce quality losses (Forney, 2003; Liew and Prange, 1994).

In addition, in several studies, treated spears showed significantly higher counts than untreated but washed controls. Hence, careful washing with clean fresh tap water may be very effective in reducing the microbial load of asparagus spears. Indeed, this has been indicated in earlier studies (Osuna et al., 1995; Simón et al., 2004; Sothornvit and Kiatchanapaibul, 2009). On the other hand, water quality cannot always be guaranteed in practise due to the

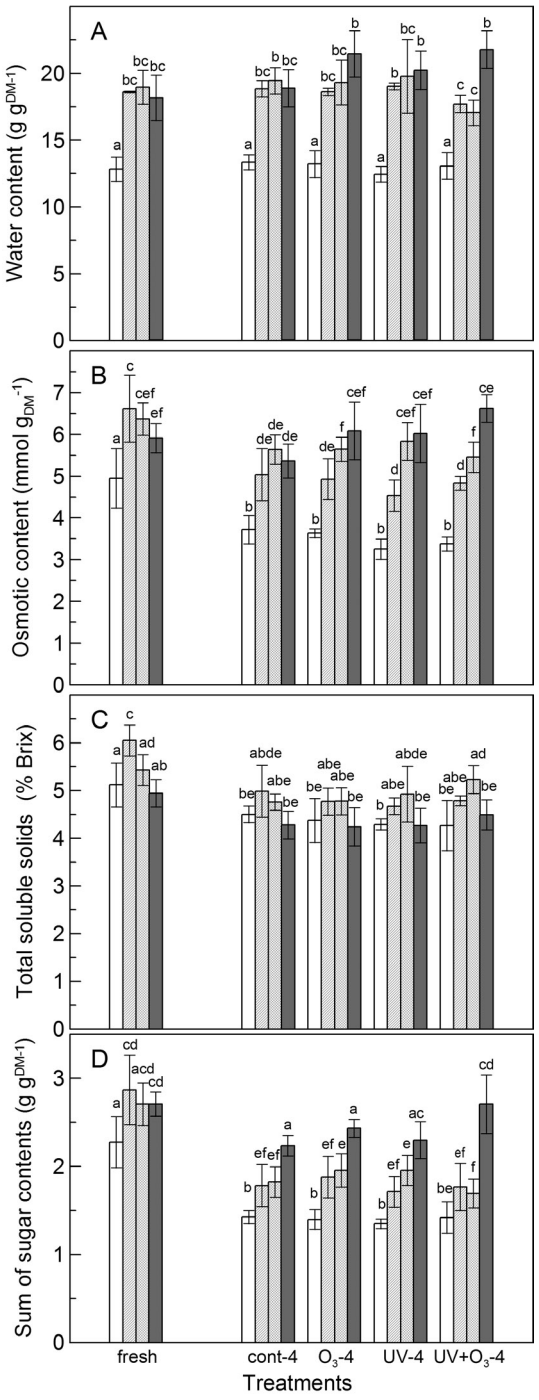

Fig. 3: Means (± SD; n ≤ 6) of water content (A), osmotic content (B), total soluble solids content (C) and the sum of the contents of all soluble sugars (D) of fresh and stored (at 20 °C in water vapour saturated air for up to four days) asparagus spears in 2006 (□ open bars) 2008 (▨ crossed bars), 2009 (▨ hatched bars) and 2011 (■ filled bars). Before storage spears were either untreated (cont), washed with ozonated water (90 s, 3 or 4.5 ppm; O_3), irradiated with UV-C (1 kJ m^{-2}; UV) or both washed with ozonated water and irradiated (O_3+UV). Different letters indicate significant differences between means (Duncan's multiple range test, $P < 0.05$).

associated high costs. This and the increased demand for safe fresh food create the need of effective sanitation.

From the practical point of view, the combination of ozonation of washing water and irradiation of washed produce with UV-C seems to be very promising according to the hurdles concept (Scott, 1989). A combination of different treatments may induce synergistic effects, as has been reported by various authors (Garzia et al., 2003; Pan et al., 2004; Sothornvit and Kiatchanapaibul, 2009; Xu and Du, 2012; Song et al., 2012). So, Xu and Du (2012) showed that combined treatment of pears with yeast antagonist and UV-C irradiation was more effective than the single treatments alone. Furthermore, washing celery and cherries with chlorine dioxide solution in combination with UV-C irradiation resulted in a better produce decontamination then single applications of each treatment (Song et al., 2012). However, these results could not be verified during the presented long-term study on white asparagus spears.

Conclusions

The initial microbial loads (mould and yeasts, and aerobic mesophilic total bacterial counts) of white asparagus spears varied considerably during the different harvest seasons of this five-year study. This variability could not be explained by the variance of climatic conditions or the place of harvest. It was also not related to the respective water or sugar contents of spears. Furthermore, there was never a clear cut relation between the initial microbial load and the growth of pathogens during four-day storage at 20 °C in nearly water vapour saturated atmosphere.

Neither washing the spears with ozonated water (3 or 4.5 ppm) nor treating them with UV-C radiation (1 kJ m^{-2}) systematically and significantly affected their microbial loads during storage. In addition, the assumption that a combination of both could synergistically improve the effect of each treatment could not be verified during the presented long-term study.

Microbial load and pathogen development in asparagus spears seem to be highly persistent and thus, other application methods of ozone or other postharvest treatments (e.g. ethanol, heat impulse techniques) meeting hygienic requirements have to be considered in further investigations.

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