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

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Summary

Optimization of postharvest treatments and storage requirements to reduce microbiological spoilage is essential for the food supply chain of asparagus. In this context, Generally Recognized As Safe (GRAS) treatments such as UV-irradiation and washing with ozonated water gain more and more importance. Information on UV-C and ozone as postharvest treatment for quality assurance of white asparagus is scanty. In the present study, asparagus spears were harvested and exposed to the above mentioned treatments and their combination. The influence of both postharvest treatments on biomechanical and biochemical textural related cell wall metabolism was investigated. UV-C-irradiation and washing with ozonated water resulted in a slight reduced respiration in white asparagus spears, but increase in spear tissue toughness. Total cell wall compounds were only tendentiously reduced after 4 days of shelf-life at 20°C by application of aqueous ozone and UV-C. However, the dosages used in this experiment were relatively low and, hence, did not have pronounced effects. Furthermore, the possible mechanism of UV-C and ozone mediated changes in textural related enzyme activities of white asparagus spears have to be investigated in more detail.

Introduction

The demand for white asparagus (Asparagus officinalis L.) has greatly increased during recent years. This is not only due to its unique flavour; asparagus is also considered a highly nutritional vegetable containing important antioxidant and anticancerogenic compounds. The fleshy spears of white asparagus are developmental immature and rapidly growing subterraneous shoots (O'DONOGHUE and SOMERFIELD, 1998). After harvest, they retain their physiological activity and even continue growth at high rates leading to a rapid decline of respiratory substrates and drastic changes in textural related cell wall metabolism (HERPPICH et al., 2005). The unaltered continuation of shoot differentiation also includes thickening and lignifications of cell walls of both sclerenchyma sheath cells and vascular bundles (WALDRON and SELVENDRAN, 1990). The spears become fibrous and though being highly undesired in horticulture. Thus, toughness of asparagus spears is a major factor in determining their quality.

As asparagus spears are purchased not only as fresh commodity but increasingly as convenience product (i.e. sliced, fresh-cut), quality assurance has to focus on one hand on the retardation of metabolic processes accompanied by undesired textural changes and, on the other hand, on the avoidance of microorganism development in postharvest, thus meeting hygienic requirements. In food industry, losses due to microbiological spoilage (e.g. *Pectobacterium caro-tovorum, Phythophthora infestans, Escherichia coli* etc.) have been estimated as being as high as 30 %. Therefore and due to new food safety regulations (HACCP concept, traceability), the optimization

of postharvest treatments and storage requirements is an essential tool for the food supply chain management of asparagus.

Postharvest treatments targeted to sanitation purposes may include physical treatments (e.g. UV-irradiation, gamma-irradiation) or fumigation treatments with Generally Recognized As Safe (GRAS) compounds such as ozone (JAMIESON et al., 2009). Sanitation treatments with chlorine or methylbromide are forbidden by law. Thus, UV-irradiation and ozone gain more and more importance. Numerous studies have already been focused on the bactericidal and fungicidal capacity of ozone and UV-C treatments, but information on product quality attributes and physiological responses is limited.

Ozone is a strong oxidizing agent that acts on carbon residues dissolved in the washing water as well as on the product surface, and thus, it is very effective in destroying microorganisms. The advantage of using ozone is the efficacy at low concentrations and short contact times as well as the absence of detectable residues in/on treated food due to the quick auto-decomposition to oxygen (JAMIESON et al., 2009). It can be applied as gas or dissolved in water and has been used to sanitizing surfaces or for water desinfection being also implemented into excisting washing processes (e.g. HASSENBERG et al., 2007; ARTÉS et al., 2009), and, thus, extend storage time of perishable food. Due to its efficient bactericidal and fungicidal properties, ozone is applied to several food products such as tomato, strawberry, grape and plum (TZORTZAKIS et al., 2007a; RODONI et al., 2010), carrot (HASSENBERG et al., 2008), apple, strawberry, lettuce (HASSENBERG et al., 2007) and cantaloupe (RODGERS et al., 2004). However, rather less attention has been paid to the impacts of postharvest ozone application on quality attributes, nutritional or health promoting composition of horticultural products. SKOG and CHU (2001) and SALVADOR et al. (2006) reported that ozone significantly improved quality and storage life of broccoli, cucumber and persimmon, respectively. KEUTGEN and PAWELZIK (2008), TZORTZAKIS et al. (2007b), and AGUAYO et al. (2006) reported similar findings, demonstrating an inhibitory effect of ozone on undesired changes of anthocyanins, phenols, ascorbic acids in some strawberry cultivars and of carbohydrates, carotenoids and phenolic compounds in tomato fruits. However, application of low ozone might even enhance secondary plant metabolites such as phenolic compounds (ARTÉS-HERNÁNDEZ et al., 2007) assumingly due to plant stress responses. Moreover, ozone treatments may also result in loss of ascorbic acid (QUIANG et al., 2005), anthocyanin content and inhibition of desired volatile compounds (PEREZ et al., 1999).

In terms of ozone-mediated changes in textural quality, only few and contradictory studies have been performed. Those studies revealed either an ozone-induced delay in fruit softening as shown for strawberry and tomato (RODONI et al., 2010; PEREZ et al., 1999), the inhibition of undesired textural related cell wall modifications i.e. in green asparagus (AN et al., 2007), or negative effects on membrane stability in carrots (LIEW and PRANGE, 1994) and enzyme inactivation associated with the loss of crispness in lettuce (ARTÉS et al., 2009).

In contrast to the limited knowledge on the impact of ozone on

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characteristic quality attributes of fruit and vegetables during storage, a comprehensive view of UV-C effects in postharvest is present. The use of UV-C irradiation in the wavelength range of 190-280 nm is primarily effective for surface decontamination of perishables. UV-C light can directly damage microbial DNA (ARTÉS et al., 2009) or act by inducing the synthesis of plant secondary metabolites that effectively block or slow spore germination in plant tissue (PERKINS-VEAZIE et al., 2008). Application of UV-C offers several advantages as it does not leave any residue, it is lethal to most types of microorganisms, and it does not require extensive safety equipment to be implemented (ARTÉS et al., 2009).

Many studies have reported the advantageous effects of low or sublethal doses of UV-C (0.2-14 kJ m⁻²) to reduce microbial growth in e.g. broccoli (LEMOINE et al., 2007), strawberry (MARQUENIE et al., 2002; PAN et al., 2004), carrots (MERCIER et al., 2000), grapefruit (D'HALLEWIN et al., 2000), processed food (BINTSIS et al., 2000), peach (EL GHAOUTH et al., 2003) and tomatoes (LIU et al., 1993). However, UV-C is also known to delay ripening-associated and postharvest senescence processes (BARKA et al., 2000; COSTA et al., 2006; GONZALEZ-AGUILAR et al., 2007; POUBOL et al., 2010), to reduce chilling injury in pepper (VICENTE et al., 2005) and to improve firmness retention (MAHARAJ et al., 1999; BARKA et al., 2000; LAMIKANRA et al., 2005; POMBO et al., 2009). Contradicitive findings are reported by ALLENDE et al. (2006) and ARTÉS et al. (2009), who found an UV-C induced tissue softening and browning in lettuce and caulifower, while no effect of UV-C on green asparagus spear texture was found (POUBOL et al., 2010). As demonstrated, UV-C contributes to alleviate and reduce tissue colonization by pathogen (e.g. PAN et al., 2004) and on the other hand, UV-C affects several physiological processes, i.e. acting indirectly by stimulating plant defence mechanism in fruit and vegetables, leading to an accelerated synthesis of secondary plant metabolites and thus, is able to enhance nutraceutical content (CISNEROS-ZEVALLOS, 2003; PERKINS-VEAZIE et al., 2008).

Nevertheless, information on physiological responses and textural properties of white asparagus as affected by ultraviolet-C (UV-C) radiation and ozonated water is scant. Moreover, the mechanism underlying the mode of action of these physical and chemical postharvest treatments on texture related cell wall metabolism is poorly understood. Hence, the aim of our investigation was to evaluate the plant responses to UV-C and ozone in terms of quality-related textural properties of asparagus spears during shelf-life.

Material and methods

Plant material and experimental setup

Freshly harvested from a commercial field (Spargelhof Nottebohm GbR, Kartzow, Germany), white asparagus spears of the cultivar Gijnlim were transported to the laboratory, washed, sorted according to EC quality standard class I, cut to a length of 22 cm (mean spear diameter: 1.8 ± 0.2 cm) and randomly separated into 27 batches of approximately 500 g. Thereafter, batches of spears were subjected to the following treatments a) UV-C (254 nm) at 1 kJ m⁻² for 8 min using a 12 W VL-6C UV-C light source (6 W-254 nm Tube, Vilber Lourmat, Marne-la-Vallee, France) or b) submerged in ozonated water (3 ppm) for 30 s or c) combined treatment of UV-C (1 kJ m⁻² for 8 min) and ozonated water (3 ppm) for 30 s. Ozonated water was generated using a "Bewazon 1" ozone generator (0.02 g O₃ min⁻¹; BWT Water Technology Ltd., Schriesheim, Germany). For all O₃-treatments, water temperature was set to 10°C using a thermostat 45 (Haake, Karlsruhe, Germay). Ozone concentration was measured with a complementary chlorine/ozone cuvette test applying a LASA® 2plus photometer (Dr. Bruno Lange GmbH & Co., Düsseldorf, Germany).

Before treatments as well as after 2 and 4 d of storage, treated and untreated (control) spears were shock-frozen in liquid nitrogen and kept at -25 °C for further analysis of cell wall composition (pectic substances, cellulose, hemicellulose, lignin). During storage, each batch of treated and control spears was placed loosely in a plastic container (30 x 40 x 5 cm²) and fully covered with cloths, which was carefully soaked with demineralised water. In this water vapour saturated atmosphere, spears were stored at temperatures of 20 °C for up to four days. The experiment was conducted with three replicates per treatment and repeated three times.

Determination of respiration and biomechanical properties

On the initial day of the experiment (day 0), 12 spears were used for measurements to evaluate the biological variability. On days 2 and 4 of the experiment, six spears per treatment (2 of each batch) were randomly taken out of storage and equilibrated to room temperature (approximately 21.4 ± 0.9 °C) in water vapour saturated atmosphere for 1 h. Then, CO₂ release of mixed samples of three asparagus shoots was measured at 20 °C in closed Perspex® cylinders with infrared sensors (FYA600CO2, Ahlborn Mess- und Regeltechnik GmbH, Holzkirchen, Germany). From the increase in CO₂ concentration over time and the spears' dry mass, potential respiration activity was calculated as mmol g⁻¹ d⁻¹.

Afterwards, fresh mass (FM, electronic balance BP 210 S, Sartorius AG, Göttingen, Germany), total length, and the diameters at positions 2.5 cm, 7.5 cm, 12.5 cm and 18 cm from the base (electronic sliding calliper) were determined for each spear. Thereafter, the spears were sliced with a stainless steel microtome blade (Feather S35, 0.26 mm total thickness) adapted to a Zwicki 1120 material testing machine (Zwick, Ulm, Germany; crosshead speed 600 mm min⁻¹) to obtain tissue strength *E* was calculated from the length of deformation at a force of 2 N (ATKINS and VINCENT, 1984; BILLAU et al., 1990; HERPPICH et al., 2005).

Mean cutting force over the entire spear diameter (F_{cut}) and the actual cutting length (L_{cut}) was used to calculate the cutting energy ($E_{cut} = F_{cut} / (L_{cut} * \pi/4)$, which is closely related to spear toughness and fibrousness (VINCENT, 1990).

From the sections, fresh mass (FM) was obtained. All sections were dried in an oven (85 °C) to constant weight for determination of the spear dry mass (DM). Dry mass related water content was calculated as $WC_{DM} = (FM - DM)/DM$.

Analysis of biochemical cell wall properties

Approximately 300 g of shock-frozen asparagus spears of each treatment were freeze-dried (Christ Alpha 1-4, Christ; Osterode, Germany), and thereafter subjected for further analysis of cell wall content of proteins, pectic substances, cellulose, hemicellulose and lignin which in total are presented in Fig. 3 as total cell wall compounds.

The alcohol insoluble fraction (AIF) was used to determine the cell wall protein content modified according to the method described by BRADFORD (1976). Fifty mg of the dried ground material was dispersed in 1 ml phosphate buffer with a vortex mixer ($3 \times 10 \text{ s}$), and afterwards centrifuged (11400 rpm, 15 min) at 4 °C. In a reaction tube, 100 µl of the supernatant were diluted with 900 µl phosphate buffer, 1 ml coomassie blue added and the carefully mixed solution measured photometrically (595 nm) after 20 min (PU 8730, Philips, Kassel, Germany). A calibration series (10 to 50 µg) was obtained from phosphate buffer and Bovine Serum Albumin.

Cell wall extraction for the determination of pectic substances (water soluble pectin, EDTA-soluble pectin and water insoluble pectin) was conducted according to BLUMENKRANTZ and ASBOE-HANSEN (1973) modified by HUYSKENS (1991). The colorimetric

300

250

200

150

100

determination of the pectin fractions was conducted using metahydroxybiphenyl (MHDP, Sigma H 6527, Sigma-Aldrich Chemie GmbH, München, Germany) as a colour reagent and following the method described by MCCOMB and MCCREADY (1952). In each fraction, the amount of galacturonic acid was measured photometrically (PU 8730, Philips, Kassel, Germany) at 520 nm, Analyses were performed with three replications for each treatment. The content of pectic substances was expressed as mg galacturonic acid g⁻¹ dry mass.

Cellulose and lignin were analysed according to the methods of GOERING and VAN SOEST (1972) and AOAC (1999). One gram freeze-dried sample was extracted with 100 ml Acid Detergent Fibre (ADF) reagent (N-Cetyl-N, N,N-trimethyl-ammonium bromide dissolved with 96 % H₂SO₄) using a Fibertec System (M 1020, Tecator, Sweden). Thereafter, the solution was vacuum filtered, washed with boiled double distilled water until removal of the acidity and again washed with 90 % acetone. The residue was dried at 105 °C for 24 h, weighed, ash-dried at 500 °C for 24 h and weighed again to calculate ADF. The dried ADF residue was used for Acid Detergent Lignin (ADL) determination. Cellulose content was calculated as the difference between ADF and ADL. The content of lignin and cellulose, respectively, were expressed as mg g^{-1} dry mass.

With the Neutral Detergent Fiber (NDL) approach (VAN SOEST and GOERING, 1963), one gram of freeze-dried material was cooked in 100 ml of NDL mixture (Titriplex III, disodium borate, dodecyl hydrogen sulphate sodium, ethylene glycol monoethyl ether) to determine the hemicellulosic cell wall fraction. Afterwards, the solution was vacuum filtered, washed with demineralized water and with 90 % acetone. The insoluble residue was dried at 105 °C for 24 h, weighed, ash-dried at 500 °C for 24 h and weighed again to calculate NDF. The hemicellulose content was obtained by subtracting ADF from NDF and given as mg g⁻¹ dry mass.

Statistical analysis

All data were statistically analysed (ANOVA) with SPSS 13.0 (SPSS Inc., USA). Significant differences were determined by Tukey test (p < 0.05). In figures, the mean variability of data was indicated by the standard deviation.

Results and discussion

Asparagus spears are known for their high postharvest metabolic activity which leads to a rapid decline of nutritionally valuable respiratory substrates such as sugars or organic acids. Moreover, the unaltered shoot differentiation also includes thickening and lignifications of cell walls in the sclerenchyma ring and in vascular bundles. These processes rapidly result in the undesired toughening of spears. In practice, these changes in postharvest spear quality are predominantly controlled by ice-cooling of harvested spears and cold storage. On the other hand, UV-C irradiation or washing with ozonated water, which may be applied for fresh produce sanitation in various European and Non-European countries, are known to potentially affect texture properties of treated produce. However, studies on the impact of these treatments on spear textural properties are rare (An et al., 2006; An et al., 2007; POUBOL et al., 2010) and information yet published on possible responses of plants to these treatments is equivocal. Therefore, this study elaborates the effects of UV-C and ozone treatment on physiological activity of white asparagus spears and focused on their impact on the texture related biochemical and biophysical properties of spears.

In the presented investigation, respiration, an indicator of overall physiological activity, continuously declined during storage. The observed changes were, however, only slightly influenced by UV-C or ozone or the combined application of both (Fig. 1). It could be



observed that both treatments tended to reduce the physiological activity of asparagus spears during shelf-life, i.e. they alleviated respirational carbon losses. Nevertheless, this effect was only limited and not significant in comparison to control spears.

There are some reports that washing with ozonated water, also in combination with UV-C, do not reveal a pronouncedly retard respiration and ethylene production, e.g. in tomatoes (TZORTZAKIS et al., 2007b) and Brassica spp. (MARTINEZ-SÁNCHEZ et al., 2008). Significant inhibition of microorganism development by UV-C and ozone was demonstrated for many horticultural commodities (e.g. TZORTZAKIS et al., 2007a; HASSENBERG et al., 2008; RODONI et al., 2010): Ozone and UV-C treatments, on the other hand, have been reported to accelerate physiological processes, thus, reducing overall produce quality. In this context, enhanced respiration may indicate metabolic disturbance due to a rising energy and substrate demand for repair processes of ozone or UV-C mediated physiological injuries (LIEW and PRANGE, 1994). For ozonation, this increase in respiration, being possibly associated with a rise in ethylene production, has been reported to be accompanied by decrease tissue and membrane stability (LIEW and PRANGE, 1994). This was obviously not the case in white asparagus spears.

In green asparagus spears, UV-C irradiation was found to accelerate respiration; this response was also accompanied by changes in spear crispness (POUBOL et al., 2010). In the present study, there was a significant increase in cutting energy and, thus, in tissue toughness of all asparagus spears within four days of shelf-life (Fig. 2). This increase could not significantly be prevented by the postharvest treatments applied. Nevertheless, washing with ozone tendentiously retarded the increase in spears toughness during prolonged storage. In contrast, UV-C irradiation and its combination with ozone resulted in an enhanced cutting energy compared to control spears on day 4 of storage. This may reflect the findings of POUBOL et al. (2010); it may point to more general shoot response to increased UV irradiation.

In addition, spear stiffness declined during the entire storage period, i.e. spears became more elastic irrespective of the treatments (data not shown). Results reported on the effects of washing with low ozone concentrations (0.05 - 1.0 µmol mol⁻¹) as well as low UV-C doses (0.5 - 4.0 kJ m⁻²) on elastic properties of fruits are highly equivocal. Low-level ozone (0.005 - 1.0 µmol mol⁻¹) as well as low UV-C (0.5 - 4.0 kJ m⁻²) applications were reported to have no pronounced effect on fruit firmness of tomato (TZORTZAKIS et al., 2007b) and blueberry (PERKINS-VEAZIE et al., 2008), while in contrast, UV-C irradiation even induced tissue deterioration in

-untreated

ozone + UV-C

- ozone

-UV-C



Fig. 2: Cutting energy (J m⁻²) of asparagus spears during storage (4 days at 20 °C in water saturated air) as affected by postharvest treatments of UV-C (1 kJ m⁻² for 8 min), ozonated water (3 ppm for 30 s) and combined UV-C and ozonated water. Data are means ±SD (n=6).

lettuce and caulifower (ALLENDE et al., 2006; ARTÉS et al., 2009). The UV-C effect, however, obviously dependent on the irradiation dosage applied. In green asparagus, low UV-C doses of up to 2.4 kJ m⁻² prevented asparagus spear toughening for 4 days of storage; in contrast application of UV-C above 3.8 kJ m⁻² did not show any effect on textural properties (POUBOL et al., 2010). This is not consistent with our results. Even the very low UV-C doses (1 kJ m⁻²) applied significantly enhanced tissue toughness in white asparagus spears.

The observed changes in white asparagus spear toughness were not due to any variation in tissue water status (data not shown; HERPPICH et al., 2005). In contrast, they have been shown to be largely associated with changes in structural cell wall components (HERPPICH and HUYSKENS-KEIL, 2008). In the presented study, however, the general increase in the cell wall components (cellulose, hemicelluloses, pectic substances, lignin and protein) in control spears (Fig. 3), was even retarded by aqueous ozone, UV-C and combined ozone/UV-C applications. This was attributed to changes in the composition of the structural major cell wall components. Whereas cellulose and hemicellulose were not influenced by the treatments (data not shown), the general increase in lignin (Fig. 4) and pectin (Fig. 5) appeared to be slightly inhibited by UV-C irradiation and the combined UV-C/ozone treatment after 4 days



Fig. 3: Changes in total content of structural cell wall compounds (mg g DM⁻¹) of asparagus spears during storage (4 days at 20°C in water saturated air) as affected by postharvest treatments of UV-C (1 kJ m⁻² for 8 min), ozonated water (3 ppm for 30 s) and combined UV-C and ozonated water. Data are means ±SD (n=6).



Fig. 4: Changes in cell-wall lignin (mg g DM⁻¹) of asparagus spears during storage (4 days at 20 °C in water saturated air) as affected by postharvest treatments of UV-C (1 kJ m⁻² for 8 min), ozonated water (3 ppm for 30 s) and combined UV-C and ozonated water. Data are means ±SD (n=6).



Fig. 5: Changes in cell-wall pectic substances (mg g DM⁻¹) of asparagus spears during storage (4 days at 20 °C in water saturated air) as affected by postharvest treatments of UV-C (1 kJ m⁻² for 8 min), ozonated water (3 ppm for 30 s) and combined UV-C and ozonated water. Data are means ±SD (n=6).

of shelf-life. At the early storage, lignin incorporation was even slightly accelerated by UV-C and ozone in comparison to control spears. UV-C irradiation was shown to activate the synthesis of compounds of the phenylpropanoid pathway, such as lignin and phenolic compounds by stimulating the activity of the key enzyme phenylalanine ammonia-lyase (PAL) (CHARLES et al., 2008). This lignification is assumed to mainly cause reinforcement of the cell wall.

On the other hand, several studies demonstrated that UV-C and ozone treatments may reduce the activity of texture-related enzymes generally enhanced during postharvest life of fruits and vegetables (e.g. COSTA et al., 2006). In green asparagus, aqueous ozone inhibited PAL activity and thus, the increase in phenolic cell wall compounds (AN et al., 2006). Furthermore, UV-C irradiation may reduce activity of cell wall degrading enzymes (e.g. PG, PME), thus delaying the disintegration of membrane and the loss of tissue firmness (e.g. BARKA et al., 2000; POMBO et al., 2009).

The degradation of cell wall components is related to the enzymatic activity of several cell wall proteins (BRUMMELL and HARPSTER, 2001). In the presented study, cell wall protein content of asparagus spears nearly linearly decreased during the experimental period.

This effect was significantly accelerated by all postharvest treatments, specifically after 4 days of storage (Fig. 6). The pronounced reduction of cell wall protein content by UV-C and ozone treatments might reflect the inhibition of PAL activity as found for green asparagus (AN et al., 2007). On the other hand, it might be an indication of stress mediated responses (KUCERA et al., 2003) associated with the loss of integrity and functionality of membranes and degradation of solubilised cell wall protein. In this context, the topic certainly requires additional systematic investigations.



Fig. 6: Changes in cell-wall protein (mg g DM⁻¹) of asparagus spears during storage (4 days at 20 °C in water saturated air) as affected by postharvest treatments of UV-C (1 kJ m⁻² for 8 min), ozonated water (3 ppm for 30 s) and combined UV-C and ozonated water. Data are means ±SD (n=6).

Conclusion

Plant reaction to hormic UV-C and ozone application is different in terms of their alarm-signalling processes. These processes serve to modify metabolism. The capacity of the antioxidative defence system, however, is often increased in response to the above treatments. As a result, radical production may exceed scavenging finally leading to disruption of metabolism as indicated e.g. by accelerated gas exchange, loss of tissue integrity and cell wall modifications. Thus, the impact of ozone and UV-C on white asparagus spears resulted in a slight reduced respiration, but increase in spear tissue toughness. Total cell wall compounds were only tendentiously reduced, while cell wall protein was strongly affected after 4 days of shelf-life at 20°C by application of aqueous ozone and UV-C. The degradation of solubilised cell wall protein might indicate stress mediated responses associated with the loss of integrity and functionality of membranes. Further emphasis will be placed on the possible mechanism of UV-C and ozone mediated changes in textural related enzyme activities of white asparagus spears in more detail.

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