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High CO_2 effects on postharvest biochemical and textural properties of white asparagus (*Asparagus officinalis* L.) spears

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ABSTRACT

The effects of high CO_2 concentration (10% CO_2, 17% O_2) on the changes of functional cell wall components (pectic substances, hemicellulose, cellulose, lignin), mechanical properties, content of free soluble sugars (sucrose, glucose, fructose), and respiration activity were studied in harvested white asparagus spears stored at 10 and 20 °C, respectively, for up to 7 d. Spears stored at 2, 10 and 20 °C in air were studied as controls, where the 2 °C condition indicated the effects of cold storage. During storage, respiration activity declined only slightly, irrespective of the CO_2 and temperature regime. Spears stored at 20 °C under both CA and normal air became less stiff and more elastic, however, tissue toughness increased significantly. Changes in toughness were associated primarily with the dynamics of lignin and cellulose, revealing a strong correlation ($r^2 = 0.81$). High CO_2 concentration inhibited the synthesis of cellulose and, to some extent, lignin accumulation at 20 °C. Additionally, elevated CO_2 inhibited the degradation of soluble carbohydrates. In contrast, slightly lower temperatures of 10 °C in combination with high CO_2 did not have a pronounced effect on changes in structural carbohydrates (lignin, cellulose, hemicellulose and pectins). The effect low temperature (2 °C) under normal atmosphere conditions resulted in the inhibition of cell wall changes in asparagus spears.

1. Introduction

White asparagus (*Asparagus officinalis* L.; Asparagaceae) is a highly nutritional (Maeda et al., 2005) and economically valuable vegetable. The spears of this monocotyledonous herbaceous perennial are succulent fleshy subterraneous shoots that grow from rhizomes. From the botanical view, they are "developmentally immature" (O'Donogue and Somerfield, 1998) and retain their high physiological activity (Heyes et al., 1998). Hence, spears show high rates of respiration (Papadopoulou et al., 2001), rapid degradation of soluble sugars (Lipton, 1990), proteins and ascorbic acid (Siomos et al., 2000), and pronounced water loss (Lipton, 1990).

Besides discoloration or microbiological decay, texture is the most crucial postharvest parameter that negatively affects quality and shelf-life of fresh and processed white asparagus spears. In a temperature-dependent manner (Rodríguez et al., 1999b; Herppich and Huyskens-Keil, 2008), spears become increasingly tough and fibrous (cf. Lipton, 1990; Siomos, 2003). These undesired changes result from further cell wall thickening (Chang, 1983; Zurera et al., 2000), increased lignification of cell walls of sclerenchyma sheath cells and of the vascular bundle elements (Billau, 1986; Waldron and Selvendran, 1990; Rodríguez et al., 1999c) or from a rapid increase in ferulic acid cross-linking of cell wall polymers (Rodríguez-Arcos et al., 2004; Jaramillo et al., 2007). All these reactions are assumed to be controlled by wounding-induced ethylene formation (Hsiao et al., 1981; Bhowmik and Matsui, 2004; Jaramillo et al., 2007). The textural changes may also simply reflect the unaltered continuation of shoot differentiation (O'Donogue and Somerfield, 1998; Herppich et al., 2005). It is also not clear whether secondary cell wall thickening is fed by a turnover of asparagus cell wall polysaccharides (Rodríguez et al., 1999c) or by a consumption of stored soluble sugars (Herppich and Huyskens-Keil, 2008).

Especially during storage or long distance transportation, low temperatures (0–2 °C) are generally recommended to retard produce quality losses and decay of asparagus (Lipton, 1990; Siomos, 2003). Low temperature diminishes water losses, reduces physiological activity and, concomitantly, respiration and consumption of soluble sugars (Chang, 1983; Lallu et al., 2000).

Because effective cool chain management is not always available during transportation and distribution (Lill and Corrigan, 1996; Renquist et al., 2005), controlled atmosphere (CA) or modified atmosphere packaging (MAP) may be successfully applied (Hurst et al., 1997; Kadau et al., 2003; Sothornvit and Kiatchanapaibul,

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2009). Comparable to low temperature, storage at high CO_2 and low O_2 is known to reduce physiological activity, thus diminishing respirational processes leading to an inhibition of energy supply for cell wall synthesis, spear toughening and changes in storage carbohydrate, protein depletion and asparagine accumulation (Everson et al., 1992; Siomos et al., 2000). However, investigations of combined CA/MAP and cold storage mostly report minor advantages over cold storage in air (Lipton, 1990; Siomos et al., 2000; Villanueva et al., 2005).

Postharvest variations in cell wall structure (Zurera et al., 2000) and composition (Redondo-Cuenca et al., 1997; O'Donogue and Somerfield, 1998; Rodríguez et al., 1999a,b,c; Kadau, 2005), and in soluble carbohydrates (Hsiao et al., 1981; Irving and Hurst, 1993; Huyskens-Keil et al., 2005; Kadau, 2005) of white asparagus spears have been investigated thoroughly. Moreover, the effects of cool and room temperature CA/MAP storage on cell wall polymers and textural properties of green asparagus have been also studied relatively extensively (e.g. Lipton, 1990; Waldron and Selvendran, 1990; Everson et al., 1992; Villanueva et al., 2005; Sothornvit and Kiatchanapaibul, 2009), but investigation on white asparagus are by far less frequent (Siomos et al., 2000; Jaramillo et al., 2007; Papoulias et al., 2009). Nevertheless, it has been shown that CA/MAP storage could largely inhibit spear toughening (Everson, 1992; Siomos et al., 2000; Villanueva et al., 2005). Variation of O_2 concentrations seems to be less effective on texture retention, but a CO_2 concentration of 15% seems to be optimal (Lougheed and Dewey, 1966). Everson et al. (1992) reported on a pronounced inhibiting effect of CA/MAP on lignin, which corresponds well with the finding that high CO_2 may probably inhibit phenylalanine ammonia-lyase (PAL, EC 4.3.1.1) (Holcroft and Kader, 1999), a key enzyme of lignin synthesis (Bhowmik and Matsui, 2004).

Despite all efforts, "the biochemistry and physiology underlying the beneficial effects of CA storage are not understood" (Hurst et al., 1997), especially concerning the effects of CA/MAP on asparagus spear toughening. It is still unclear whether CA inhibits singular physiological reactions or whether their decline is a response to a more general metabolic depression (Hurst et al., 1997). To better understand the underlying physiological processes, we studied the effects of high CO_2 concentration (10% CO_2, 17% O_2) during storage on potential respiration, content of free soluble sugars (sucrose, glucose, fructose) and the functional cell wall components (pectin, hemicellulose, cellulose, lignin) in spears stored at 10 °C (mimicking transport conditions) and 20 °C (simulating retail conditions), respectively, for up to 7 d. Asparagus stored at 2, 10 and 20 °C in air were also studied as controls (10 °C, 20 °C) and to indicate the effect of cold storage (2 °C) on biochemical and mechanical properties of white asparagus spears. This approach facilitates the comprehensive analysis of texture-related biomechanical and physiological targets in metabolic responses of this produce to high CO_2.

2. Materials and methods

2.1. Plant material and experimental design

Freshly harvested from a commercial field (Erzeugergruppierung Beelitz Spargel, Spargelgut Dietersdorf, Germany), white asparagus (*A. officinalis* L.) 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 batches of approximately 500 g. Each batch of spears was placed loosely into a plastic container (30 cm × 40 cm × 5 cm) and fully covered with cloth, which was carefully soaked with demineralised water. In this water vapour saturated atmosphere, the spears were stored at 2 °C, 10 °C and 20 °C in air (0.03% CO_2 and 21% O_2), and at 10 °C and 20 °C in controlled atmosphere (10% CO_2, 17% O_2) for up to 7 d (3 repetitions, i.e. 3 batches each of approx. 500 g per day per treatment). For rapid and continuous CA adjustment, climate chambers (SB222, Weiss Umwelttechnik GmbH, Balingen, Germany) with a gas mixing unit and Ultramat CO_2/O_2 controller (Siemens AG, München, Germany) were used. Two independent experiments were performed.

2.2. Determination of respiration and mechanical properties

On the initial day of each experiment (day 0), 12 spears were used to evaluate the initial biological variability. On days 2, 4 and 7 of the experiment, six spears per treatment (two spears per batch) were randomly taken out of storage, equilibrated to room temperature (approximately 21.4 ± 0.9 °C) in water vapour saturated atmosphere for 1 h. Then, CO_2 release of all six asparagus shoots (on day 0 three mixed samples of four spears) was measured at 20 °C in closed Perspex cylinders with infra red sensors (FYA600CO2, Ahlborn Mess-und Regeltechnik, Germany). From the increase in CO_2 concentration over time and the spears' dry mass, respiration activity was calculated as mmol CO_2 g_{DM}^{-1} d^{-1}. Afterwards, fresh mass (*FM*, electronic balance BP 210 S, Sartorius AG, Göttingen, Germany), total length, and 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.

The acoustic impulse-response technique (Herppich et al., 2005) was then applied to determine the dynamic stiffness coefficient (*S*). Induced by slightly striking the spears with a little hammer in the middle section, the resulting sound signal was recorded with a microphone connected to the soundcard of a laptop (10 measurements per spear). From the first local maximum frequency (*f*) of the frequency spectrum, obtained after fast Fourier transformation of the raw sound signal, and the respective spear fresh mass, *S* was calculated as $S = f^2\ FM^{2/3}$.

Finally, on the positions 2.5, 7.5, 12.5 and 18 cm from base, the spears were sliced with a stainless steel microtome blade (S35, 0.26 mm total thickness, Feather Safety Razor Co. Ltd., Osaka, Japan) adapted to a Zwicki 1120 material testing machine (Zwick, Ulm, Germany; crosshead speed 600 mm min^{-1}) to obtain tissue toughness (Atkins and Vincent, 1984; Herppich et al., 2004), which is closely related to spear fibrousness (Vincent, 1990). Mean cutting force over the entire spear diameter (F_{cut}) and the actual cutting length (L_{cut}) were used to calculate the cutting energy ($E_{cut} = F_{cut}/(L_{cut} * \pi/4)$).

2.3. Analysis of carbohydrate content and chemical cell wall properties

On days 0, 2, 4 and 7, approximately 300 g of asparagus spears of each treatment were removed from the storage, freeze-dried, and thereafter subjected for further analysis of soluble mono- and disaccharides, and cell wall content of proteins, pectic substances, cellulose, hemi-cellulose and lignin.

After hot (70 °C) extraction of ground freeze-dried material (100 mg, three replicates) with ethanol (80%), the mono- and disaccharides (glucose, fructose and sucrose) were determined by High Performance Liquid Chromatography (HPLC Model 250, with RI-detector, 8110, Bischoff, Germany and Autosampler 708, Alcott, USA) over a water-spherisorb-amino column (250 mm × 3.0 mm, Bischoff, Leonberg, Germany) according to Ulrichs (1999). The mobile phase was acetonitrile and water (85:15) with a flow rate of 1 mL min^{-1}. Standard carbohydrate solutions (glucose 49140, Fluka, Neu-Ulm, Germany; fructose 5323, Merck KGaA, Darmstadt, Germany; sucrose 716260, Böhringer, Ingelheim, Germany) were prepared. The content of mono- and disaccharides was expressed as mmol g^{-1} dry mass.