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Effects of short-term low-temperature storage on mechanical and chemical properties of white *Asparagus* cell walls

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Summary

White asparagus spears, which are very perishable horticultural products, are normally stored at low, non-freezing temperatures to prevent quality losses for few days. On the other hand, low non-freezing temperatures may induce cold-acclimation responses in many perennial plants. This may affect the mechanical and chemical attributes of the products' texture. For fresh asparagus spears texture is the predominant but also very complex quality indicator. To address this complexity in detail, the dynamics and interactions between mechanical texture characteristics, water status, and physical and chemical cell wall properties were investigated in white asparagus spears during short-time storage at different storage temperatures. Tissue stiffness as indicated by both the apparent quasi-static elastic modulus *E* and the dynamic acoustic stiffness *S*, declined at storage temperatures of 0°C and 20°C, whereas they remained nearly constant at 5°C and 10°C. Changes in pressure potential closely reflected the variation in tissue stiffness. These changes in elastic properties paralleled an increase in water insoluble pectic substances. In contrast, tissue strength of asparagus spears, which was determined as the mean radial cutting force, declined at all storage temperatures after two days of storage. This decline, which was more pronounced at 0°C and 5°C, correlated with a decrease in the EDTA soluble pectin fraction and with the increasing water potential. This increase in water potential occurred without changes in water content but resulted from the rapid decline in the osmotic content partially due to a reduction in glucose content. In contrast, the storage treatments did not affect fructose and sucrose concentration but significantly induced *myo*-inositol accumulation especially in spears stored at low temperatures. In contrast to asparagus roots no indications of cold acclimation or drought stress response occurred in stored spears. Hence, the variation in tissue texture seems to result from developmentally regulated chances in cell wall properties.

Introduction

White asparagus is an economically valuable and highly demanded fresh vegetable. In Germany, it is one of the most important vegetable crops comprising approximately 14% of the whole vegetable production (ZMP, 2002). On the other hand, white asparagus spears are extremely perishable produce „that should not be stored for more than 1 day" (CHANG, 1983). However, rapid pre-cooling and storage at low, non-freezing temperature (HERNÁNDEZ-RIVERA et al., 1992) is widely used to increase the keeping quality of white asparagus spears (VOGEL, 1996). Low temperature storage may, in general, reduce the very high physiological activity of this „developmental immature, rapidly growing" shoot (O'DONOGHUE and SOMERFIELD, 1998).

As a consequence, low temperature decreases the exceptionally high respiration activity of the spears (PAPADOPOULOU et al., 2002) and diminishes the degradation of soluble sugars (LIPTON, 1990; SCHEER et al., 2003), proteins and ascorbic acid (SIOMOS et al., 2000), and diminishes the risk of water losses (LIPTON, 1990). Furthermore, it partially inhibits spear toughening (LIPTON, 1990; SIOMOS et al., 2000). The increase in toughness, which is normally attributed to an enhanced fibre content and degree of lignification during „long-term" (i.e. within weeks) storage (WALDRON and SELVENDRAN, 1990), is a major undesired postharvest effect in white asparagus spears (SIOMOS et al., 2000). Therefore, it is usually recommended to store white asparagus spears at 0°C to 2.5°C in a high humidity (95%) atmosphere (LIPTON, 1990; SIOMOS, 2003).

On the other hand, it has been reported that storage at or only slightly above 0°C causes symptoms of chilling injury in white asparagus spears (LIPTON, 1990; SIOMOS, 2003). This is surprising because asparagus plants are known to be partially frost hardy (ARORA et al., 1992). Furthermore, it has been shown that Asparagus seedlings largely increase their freezing hardiness when they were exposed to 3°C for some days (BURROWS et al., 1989). Hence, it is reasonable to assume that low, non-freezing temperature may induce cold-acclimation responses in growing asparagus plants and, if so, also in harvested young shoots i.e. spears.

Observed in many perennial plants of temperate origin, cold acclimation or cold hardening is essential for over-winter survival of the persisting plant parts (GRAHAM and PATTERSON, 1982). Regulated at the gene expression level (THOMASHOW, 1999; XIONG et al., 2002) it includes numerous physiological alterations such as changes in membrane lipid composition (DANYLUK et al., 1998) and increased concentrations of sugars, soluble proteins, amino acids and organic acids (SVENNING et al., 1997; STRAND et al., 1999). The latter responses, summarized by the term osmotic adjustment, may diminish the risk of freezing by decreasing the actual freezing point of the cell sap (HERPPICH et al., 2000, 2001a, b). Cold hardening also includes anatomical (RAPACZ, 2002) and histological adjustments (STRAND et al., 1999) and affects the mechanical properties (RAJASHEKAR and LAFTA, 1996; STEFANOWSKA et al., 1999; GÓMEZ et al., 2005) and the chemical composition (RENAULT and ZWIAZEK, 1997; KUBACKA-ZĘBALSKA and KACPERSKA, 1999) of cell walls, resulting in elastic adjustment (WEISZ et al., 1989; NEUMANN, 1995). Stiffening of the cell walls due to increased cell wall thickness and strength is assumed to enhance freezing protection by enhancing the resistance to extra-cellular ice formation and the ability to super cool cellular water (STEFANOWSKA et al., 1999). All these low-temperature induced changes are relatively short-termed responses occurring within few days (HERPPICH et al., 2001b).

Storage of white asparagus spears at low, non-freezing temperature may mimic natural climatic conditions inducing cold-acclimation responses in the harvested produce including both osmotic and elastic adjustment. Thus, cold-acclimation during cold storage may potentially influence produce internal quality and textural properties others than fibre accumulation or lignification-related toughening. However, the effects of low temperature on short-term changes in produce mechanical attributes and chemical cell wall properties, related to post-harvest quality of white asparagus have not yet been investigated in detail.

Furthermore, a great part of our knowledge of postharvest changes in textural quality has been derived from the investigation of the cell wall properties of green asparagus spears (WALDRON and SELVENDRAN, 1990). It is well known that there are large differences in the postharvest responses between green and white asparagus spears (CHANG, 1981; HSIAO et al., 1981; LIPTON, 1990). Hence, this important aspect of produce quality needs further comprehensive investigation.

Therefore, the effects of different storage temperatures (0°C, 5°C and 10°C, and 20°C as control) on cold acclimation-related quality attributes of white asparagus spears (water status, stiffness, tissue strength, cell wall carbohydrates) were studied. The aim was to characterise the physiological and biochemical bases of the potential cold-induced modifications in white asparagus spears stored non-wrapped under water vapour saturated conditions for up to five days.

Materials and methods

Experimental design

White asparagus spears of the cultivar *Gijnlim* were harvested from a commercial field (Erzeugergruppierung Beelitz Spargel, Klaistow, Germany) in May 2003 and immediately transported to the laboratory. The spears were gently washed, sorted according to the criteria of the EC quality standard class I, cut to a length of 22 cm (mean spear diameter: 1.82 ± 0.36 cm) and randomly separated into patches of approximately 500 g. Each patch of spears was placed loosely into a plastic container (30 x 40 x 5 cm) that was fully covered with cloths carefully soaked with demineralised water. In this water vapour saturated atmosphere the spears were stored at air temperatures of 0°C, 5°C, 10°C and 20°C (3 repetitions per temperature) for up to 4 days. After 2 days, one patch per low temperature treatment was removed from cold storage and kept at 20°C for additional 2 days to investigate shelf life under simulated retail conditions.

Biometrics, mechanical properties, water relations

On the first experimental day (day 0) six spears were used for the tests to evaluate the initial biological variability. On days 2, 3 and 4 of the experiment, three spears per treatment were randomly taken out of storage, equilibrated to room temperature (approximately 21.4 ± 0.9°C) in water vapour saturated atmosphere for 1 h before further experimentation. For each spear, fresh mass (*FM*, electronic balance BP 210 S, Satorius AG, Göttigen, 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 (electronic sliding calliper).

Then, the acoustic impulse-response technique (CHEN, 1996; HERPPICH et al., 2003) was 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 $S = f^2\ FM^{2/3}$. Afterwards, on 4 positions (2.5, 7.5, 12.5 and 18 cm from base) of each spear the quasi-static elasticity modulus (*E*; ASAE, 1999) was determined by non-destructive compression tests (v = 10 mm min^{-1}) using a Zwicki 1120 material testing machine (Zwick, Ulm, Germany) fitted with a spherical steel body (diameter = 6.35 mm). According to the formulation given by MOHSENIN (c.f. ASAE, 1999) *E* was calculated from the length of deformation at a force of 2 N.

At the same positions as the force-deformation measurements, tissue strength was finally obtained by slicing the spears (crosshead speed 600 mm min^{-1}) with a stainless steel microtome blade (Feather S35, 0.26 mm total thickness) adapted to the material-testing machine (ATKINS and VINCENT, 1984; BILLAU et al., 1990). The blades were often used and, therefore, the first cuts were discarded. Mean cutting force over the entire spear diameter was used to indicate tissue strength (HERPPICH et al., 2002, 2004). Strength is closely related to spear toughness and fibreness (VINCENT, 1990) though the method used may prevent the errors introduced by compression and extrusion (BILLAU et al., 1990; LIPTON, 1990).

Water potential and osmotic potential (after freezing/thawing) of asparagus spears tissue discs (8 mm diameter and 2 mm thick), obtained from the middle sections with a cork borer, were measured psychrometrically (VON WILLERT et al., 1995) using 9 Wescor C-52 dew point hygrometer champers connected to a Wescor HR-33T micro voltmeter via a PS-10 switchbox (Wescor Inc., Logan, USA). Pressure potential (turgor) was calculated from the difference of water potential and osmotic potential. From the remaining material of these middle sections tissue sap was extracted by freezing/thawing procedure. This sap was analysed for total soluble solid content (PR 1 digital refractometer, Leo Kuebler GmbH, Karlsruhe, Germany) and osmotic content (VAPRO 5520, Wescor Inc., Logan, UT, USA). All sections were dried in an oven (85°C) to constant weight for determination of the spear dry matter.

Analysis of carbohydrate content and chemical cell wall properties

On day 0, 2, 3 and 4, approximately 300 g fresh asparagus spears of each temperature treatment were removed from the storage, freeze-dried, and thereafter subjected for further analysis of mono- and disaccharides, pectic substances and lignin.

The mono- and disaccharides (glucose, fructose and sucrose) were determined by High Performance Liquid Chromatography (HPLC Model 250, Bischoff, Germany) according to a modified method described by ULLICHS (1999). HPLC was equipped with a RI-detector (8110, Bischoff, Germany) and an autosampler (708, Alcott, USA). A water-spherisorb-amino column (250 mm x 3.0 mm, Bischoff, Germany) was used. The mobile phase was acetonitrile and water (85:15) with a flowrate of 1.0 mL min^{-1}. Standard carbohydrate solutions (glucose 49140, Fluka, Germany; fructose 5323, Merck, Germany; sucrose 716260, Böhringer, Germany) were prepared. Analyses were conducted on 100 mg freeze-dried samples of each treatment and performed in three replicates. The content of mono- and disaccharides was expressed as mol kg^{-1} dry mass.

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 colourimetric determination of the pectin fractions was conducted using meta-hydroxybiphenyl (MHDP, Sigma H 6527, 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, 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.

Lignin was analysed according to the method 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-ammoniumbromid dissolved with 96 % H_2SO_4) using a Fibretec 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 lignin determination. The content of lignin was expressed as % Acid Detergent Fibre (ADF).

Statistical analysis

All data were statistically analysed (ANOVA) with either SPSS or WinSTAT (R. Fitch Software, Staufen, Germany). Significant differences were determined by the Duncan's multiple range test ($P < 0.05$). The mean variability was indicated by the standard deviation.

Results

In spears stored at room temperature (20°C) in a water-saturated air, the average shoot elasticity rapidly and significantly increased. Both quasi-static elastic modulus *E* and dynamic acoustic stiffness *S* declined to a more or less constant level within two days of storage under such conditions. At very low temperature (0°C), *E* and *S* also decreased, although to a lesser extent and with a different dynamic. In this case changes became significant only on the fourth day of storage. In contrast, elastic properties remained more or less constant when spears were stored at 5°C or 10°C (Fig. 1A, B). On the other hand, simulated shelf life after two days of cold storage tended to reduce stiffness in all spears (Fig. 1 A, B; closed symbols).

The physical bases of *E* and *S* as well as of their determination greatly differ (LANDAHL et al., 2004) and they were measured independently but on the same spears. Hence, the close correlation between both stiffness parameters (Fig. 2) points out that the variation in stiffness observed in the stored spears was meaningful. Furthermore, it also indicates that the average spear stiffness obtained from repeated force-deformation measurements on different spear position yields the same results as the dynamic acoustic stiffness *S*, which *per se* reflects the response of the overall product (CHEN, 1996).

In contrast to the elastic properties, spear strength declined only after the second day in storage (Fig. 1C). Average cutting force decreased further in spears stored at 0°C and at 5°C, but tended to increase again in those spears that have been stored at 20°C and 10°C. During the simulated two day post storage period at 20°C, average tissue strength also declined to nearly the same degree as in those spears kept at low temperatures; except for the 5°C treatment.

Both elastic properties and tissue strength are normally strongly positively affected by the product water content and water potential (HERPPICH et al., 2004; LANDAHL et al., 2004). However, the average spear water content did not change irrespective of the temperature treatment (Fig. 3A). This clearly indicates that storage atmosphere was indeed always water vapour saturated thus, effectively preventing any undesired water losses. Furthermore, the water potential tended to increase during the course of the experiment under nearly all storage conditions (Fig. 3B) as a result of the increase in the osmotic potential (Fig. 3C). These changes were especially pronounced in asparagus spears stored at room temperature. In these samples

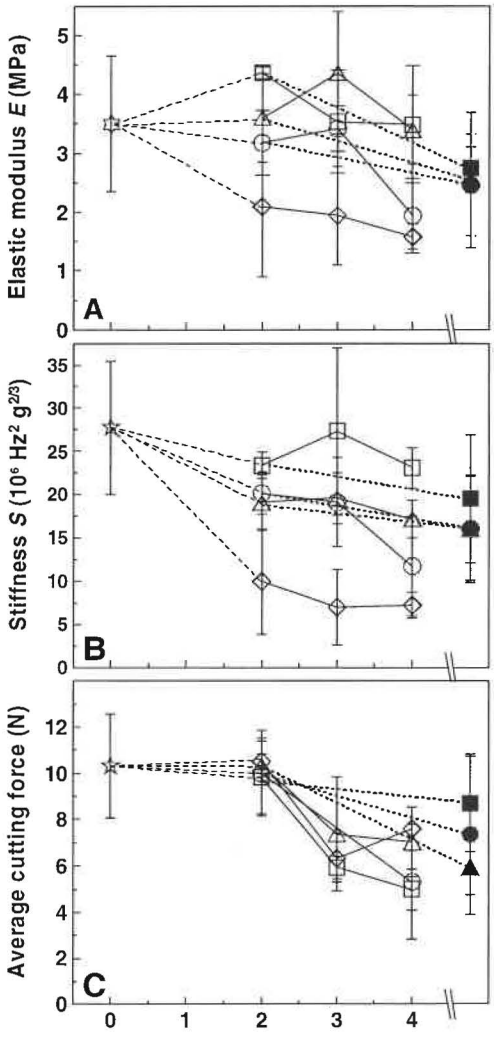

Fig. 1: Quasi-static elastic modulus *E* (A), the dynamic acoustic stiffness *S* (B) and the average cutting force (C) of fresh asparagus spears (stars; mean ± SD, n=6) and of spears stored at 0°C (circles), 5°C (squares), 10°C (triangles) or 20°C (diamonds) in water saturated air (mean ± SD, n=3). Closed symbols denote the results obtained on spears that had been removed from cold store on day 2 and kept at 20°C for additional 2 days.

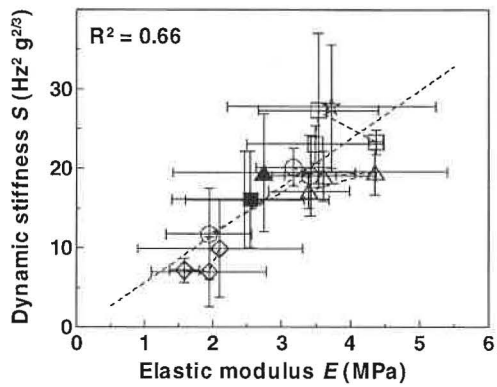

Fig. 2: Relationship between dynamic acoustic stiffness *S* and quasi-static elastic modulus *E* obtained in fresh asparagus spears (stars; mean ± SD, n=6) and in spears stored at 0°C (circles), 5°C (squares), 10°C (triangles) or 20°C (diamonds) in water saturated air (mean ± SD, n=3), and in spears that had been removed from cold store on day 2 and kept at 20°C for additional 2 days (closed symbols).

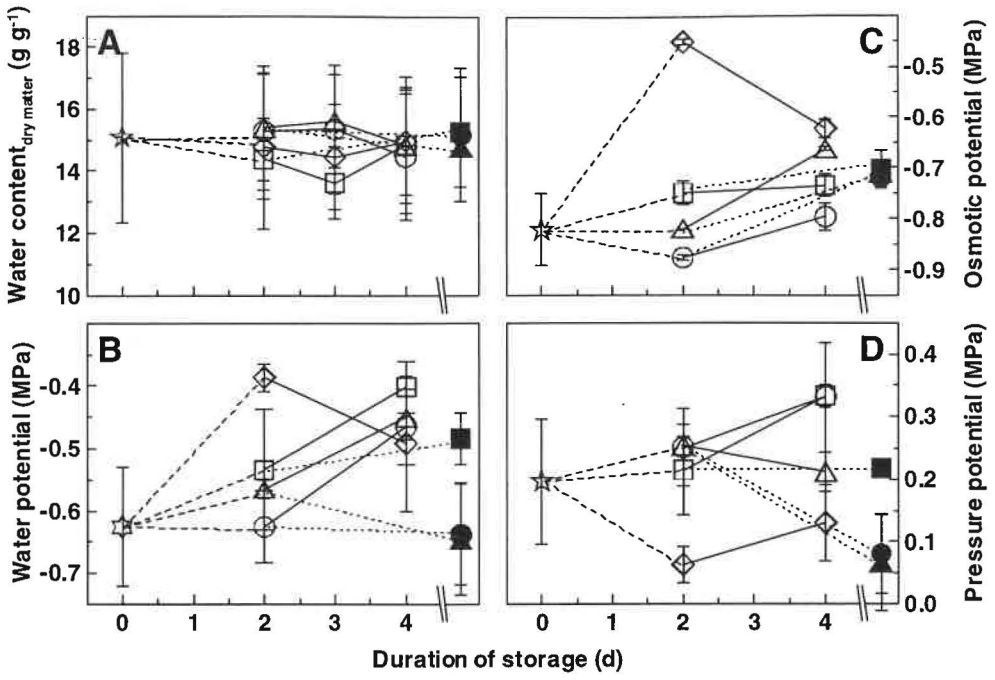

Fig. 3: Dry matter based water content (A), water potential (B), osmotic potential (C) and pressure potential (D) of fresh asparagus spears (star; mean ± SD, n=6) and during short-term storage temperatures of 0°C (circles), 5°C (squares), 10°C (triangles) or 20°C (diamonds) in water saturated air (mean ± SD, n=3). Closed symbols denote the results obtained on spears that had been removed from cold store on day 2 and kept at 20°C for additional 2 days.

changes in osmotic potential finally let to a reduction of pressure potential (Fig. 3D). On the other hand, pressure potential and, hence turgor, tended to increase in low temperature (0°C and 5°C) stored asparagus spears towards the end of the experiment. In spears exposed to room temperature after a cold storage treatment pressure potential decreased to the same level as observed in shoots continuously stored at this temperature.

As expected from the relative constancy of water content, the variation in osmotic potential seems to result from changes in osmotic content (Fig. 4A). Mean osmotic content of spears stored at 20°C rapidly declined by approximately 10% within the initial two days but slightly increased again to the same level measured in spears stored at either 5°C or 10°C. In contrast, storage at 0°C obviously prevented any changes in the osmotic content. Variation in the content of soluble sugars seems to be restricted to that of glucose, the primary substrate of glycolysis (Fig. 4C). The content of this reducing sugar tendentiously declined during the entire experiment. It finally contributed by approx. 60% to the small overall changes (0.18 mol kg_{DM}^{-1}) in total osmotic content. In contrast, the low sucrose content (Fig. 4B) and also the fructose content (Fig. 4D) seemed to be unaffected by the storage treatments. The contents of both soluble sugars significantly declined only after prolonged storage at 20°C. In the asparagus spears no starch could be detected.

myo-Inositol could not be detected in freshly harvested spears but occurred in spears of all treatments reaching a more or less constant content within two days of storage (Fig. 5). Spears stored at room temperature and those kept at this temperature after cold storage showed a weak tendency of a reduced *myo*-inositol content towards the end of the experimental period. Room-temperature storage generally led to lower *myo*-inositol contents in asparagus spears than low temperatures.

In addition to the more indirect water status mediated effect storage temperature also affects the mechanical tissue properties by a direct impact on cell wall metabolism resulting in changes in chemical cell wall composition. In this context, the content of water-soluble pectin remained more or less constant during the four days of storage (Fig. 6A). Furthermore, it seemed to be nearly unaffected by the different storage temperatures. In contrast, the cell wall content of EDTA-soluble pectic substances decreased significantly in spears stored at 0°C and 5°C during the entire experimental period (Fig. 6B), closely reflecting the decline in cutting force (cf. Fig. 1C). However, EDTA-soluble pectic substances and tissue strength seemed to increase finally again when spears were stored at higher temperatures of 10°C and 20°C (Fig. 6B, Fig. 1C). The water insoluble pectic substances significantly increased, irrespective of the storage temperature. Nevertheless, the changes were clearly most pronounced in spears stored at 10°C (Fig. 6C). This increase was associated with the loss in spear elasticity and assume an embedded layer of insoluble pectin in the middle lamella. The lignin cell wall content of spears stored at 10°C similarly increased during the entire experiment, but it tended to decline at the other storage temperatures (Fig. 6D).

Discussion

Storage temperature clearly affected various aspects of produce quality of white asparagus spears, however, to a very differentiated degree. It seems obvious that low temperatures help to retain high potential physiological activity and the nutritional value better than storing the spears in water vapour saturated air at 20°C (LIPTON, 1990; SIOMOS, 2003). Nevertheless, storage under water vapour saturated conditions evidently prevented pronounced changes in spear water content, and, hence, loss of texture even at 20°C. This reflects the well-known advantageous function of an effective high-humidity storage (HEYES et al., 1998) or protecting packaging (HUYSKENS-KEIL and KADAU, 2003; SCHEER et al., 2003). On the other hand, high humidity storage could not prevent a small relative decline in

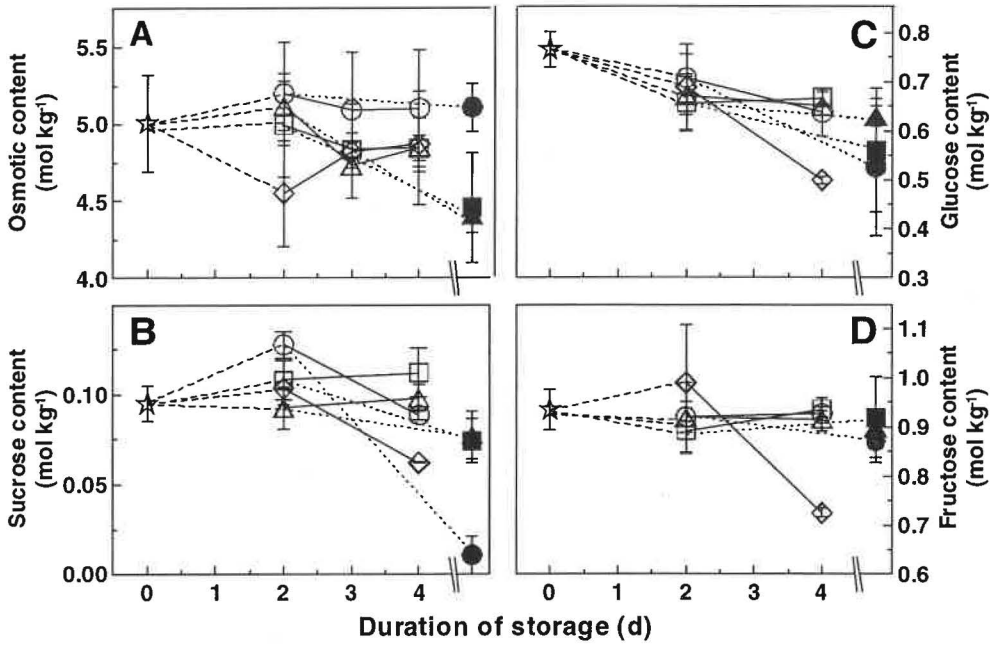

Fig. 4: Dry matter based osmotic content (A), and sucrose (B), glucose (C) and fructose content (D) of fresh asparagus spears (star; mean ± SD, n=6) and during short-term storage at temperatures of 0°C (circles), 5°C (squares), 10°C (triangles) or 20°C (diamonds) in water saturated air (mean ± SD, n=3). Closed symbols denote the results obtained on spears that had been removed from cold store on day 2 and kept at 20°C for additional 2 days.

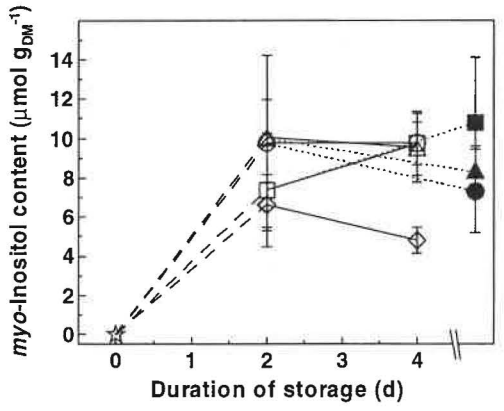

Fig. 5: *myo*-Inositol content of fresh asparagus spears (star) and from those stored at 0°C (circles), 5°C (squares), 10°C (triangles) or 20°C (diamonds) in water saturated air (mean ± SD, n=3) for up to 4 days. Closed symbols denote the results obtained on spears that had been removed from cold store on day 2 and kept at 20°C for additional 2 days.

mean spear turgor as was also shown for green asparagus (HEYES et al., 1998). However, this decline in pressure potential was due to a reduced osmotic content of the spears resulting in an increased osmotic potential at constant water volume.

As expected from the results of investigations with other crops (e.g. HERPPICH et al., 2004; LANDAHL et al., 2004) the reduction in pressure potential is accompanied by a reduced average stiffness of spears stored at 20°C. Hence, our results confirm the data presented by HEYES et al. (1998) but contradict evidence given by RODRIGUEZ-ARCOS et al. (2002a, b). These authors found a minor but significant increase in tensile stiffness of excised green and white asparagus tissue when stored at 21°C for 3 days. However, in their study no information on possible changes in tissue water relations was given.

Furthermore, we compared the variation of the average stiffness of entire spears. The close correlation of the quasi-static elastic modulus and the dynamic stiffness coefficient indicate that this approach yields meaningful results. Although these parameters were derived from very different measurements, they are both valuable indicators of produce elastic properties (LANDAHL et al., 2004). Furthermore, to our knowledge, this is the first report of the successful use of the acoustic impulse-response technique on cylindrical products such as asparagus spears. Up to now, it was exclusively applied to more or less spherically shaped objects like apples and peaches (CHEN et al., 1996). Despite the somewhat more complex resonance spectra the effective validation of this method with the standard force-deformation approach (ASAE, 1999) indicate the general usefulness of the acoustic impulse-response technique.

In contrast to stiffness, average spear strength declined only after the second day of storage, irrespective of the temperature regime used. In other investigations (LIPTON, 1990; SIOMOS et al., 2000; RODRIGUEZ-ARCOS et al., 2002a, b; BHOWMIK et al., 2003; SCHEER et al., 2003), it has been shown that the toughness of green and white asparagus spears, as measured with the shear press technique, increased during room temperature storage (approx. 20°C). According to HEYES et al. (1998) the increase in tissue strength observed in asparagus spears within one day of storage at 20°C may result from cell shrinkage rather than from cell wall toughening. In this context, it has been known since long that immersion of spear butts in water or storage in water vapour saturated air may prevent toughening or even reduces toughness (LOUGHEED and DEWEY, 1966; CLORE et al., 1976; LIPTON, 1990). Hence, it may well be that a somewhat incomplete air humidity control in other studies may be the reason for the observed discrepancy. Nevertheless, a minor increase in strength may be visible in spears stored at 20°C and 10°C, respectively, relative to those kept at 0°C and 5°C, after 4 days of storage.

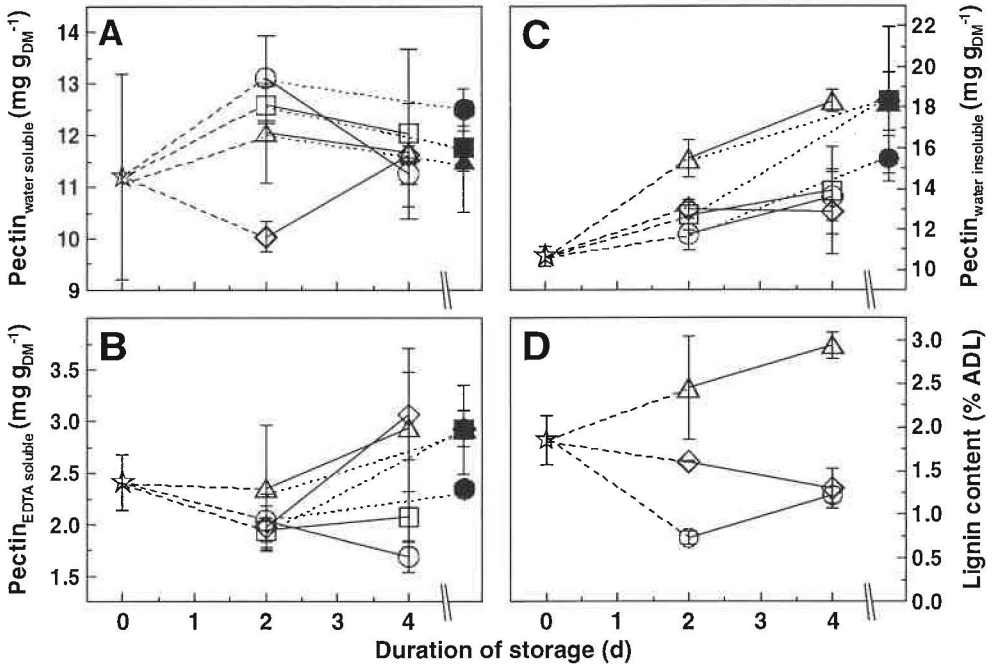

Fig. 6: Water-soluble (A), ETDA-soluble (B) and water-insoluble pectin fractions (C) of cell walls isolated from fresh asparagus spears (star) and from those stored at 0°C (circles), 5°C (squares), 10°C (triangles) or 20°C (diamonds) in water saturated air (mean ± SD, n=3) for up to 4 days. Closed symbols denote the results obtained on spears that had been removed from cold store on day 2 and kept at 20°C for additional 2 days. Changes in the cell-wall lignin contents of the same spears are shown in (D).

Low temperature storage of white asparagus spears at 5°C and 10°C could effectively prevent the decline in stiffness over the entire duration of the experiment. This was in close correlation with turgor maintenance in spears stored at these low storage temperatures. Hence, these results stress the prominent effect the mechanical component of water potential has on spear tissue elastic properties but only to a much lesser extent on tissue strength as recently shown for radish and carrots (HERPPICH et al., 2004).

On the other hand, changes in spear toughness or strength have been shown to be directly connected to a variation in fibre content (LIPTON, 1990), i.e. it has a strong relationship to the cell wall metabolism (HUYSKENS-KEIL et al., 2001; SCHREINER et al., 2002; WIDAYAT et al., 2003). The increase in portion of the pericyclic fibres (LIPTON, 1990) and xylem vascular bundles (ZURERA et al., 2000) as well as the pronounced thickening of their cell walls is primarily responsible for toughening. Although the overall contribution of pectic substances to the total cell wall material is reduced during secondary wall thickening (WALDRON and SELVENDRAN, 1990; ZURERA et al., 2000), the relative contribution of the different functional groups of pectins is still significant but differ from the primary wall status (WALDRON and SELVENDRAN, 1990; RODRIGUEZ et al., 1999b, c; MELLEROWICZ et al., 2001). In our investigation, the content of water-soluble pectin remained more or less constant during the four days of storage and was nearly unaffected by the different temperature regimes. This inhibition of the extractability of water-soluble polysaccharides was also reported by WALDRON and SELVENDRAN (1990) for long-term stored green asparagus. However, the EDTA-soluble pectic substances, which made up only a small fraction of the total pectic content, decreased significantly by 30% and 25% at low temperatures of 0°C and 5°C, respectively. This decline in the EDTA fraction closely reflected the decline in average spears cutting force in the spears of these treatments. The results may indicate a weakening of cell walls (MELLEROWICZ et al., 2001) or loss of tissue integrity when Ca^{2+} is released from the pectin bridges (WEHR et al., 2004). Hence, this might lead to the lower tissue strength instead of a high turgescent tissue. O'DONOGHUE and SOMERFIELD (1998), who reported a similar change in the content of pectic substances, concluded from their results that these changes are not senescence related.

On the other hand, the water-insoluble pectic substances significantly increased irrespective of the storage temperature. However, the changes were most pronounced in spears stored at 10°C (70% increase, compared to approx. 30% increase in the other treatments). This increase may be associated with the constancy of spear stiffness and the greater strength of cell walls of these spears compared with those stored at the other temperature. Changes in this pectic fraction may reflect an embedded layer of insoluble pectin in the middle lamella (WALDRON and SELVENDRAN, 1990). HUYSKENS-KEIL et al. (2001) reported similar results for radish. It might also indicate a continuous accumulation of galactose-enriched pectins (RODRIGUEZ et al., 1999b) of a low degree of methylation (MELLEROWICZ et al., 2001) in the thickening cell walls. In a complex with xylans these pectins might provide the initial basis for the onset of lignification (RODRIGUEZ et al., 1999c).

In this context, total lignin cell wall content actually increased in spears stored at 10°C, however, it declined at the other storage temperatures. Hence, there was no obvious relationship between mechanical properties and lignin content in our investigation. This conclusion may contradict the findings of HSIAO et al. (1981). These authors indicated that an increase in spear toughness was mainly due to an increase in lignification but not a result of fibre accumulation. The fact that inhibition of lignin synthesis by glyphosate application may reduce toughening (SALTVEIT, 1988) seems to support this hypothesis. On the other hand, this treatment also resulted in a significant reduction of total fibre content. In addition, the findings of RODRIGUEZ et al. (1999b) that toughening of asparagus spears is not directly related to the increase in lignin content nor does it depend on the absolute lignin content nicely reflects our

results. Furthermore, changes in toughness did not correlate with those of total peroxidase activity being known as an indicator of lignification potential (RODRIGUEZ et al., 1999a). These authors found an increase in the cellulosic fraction while there was a decrease in the extractability of soluble polysaccharides, before toughness increased. It seems therefore highly probable that the effect of lignin on spear toughening is only a secondary response, which is closely and directly linked to the increased fibre content, i.e. an increase in secondary cell wall material as the basis for lignification.

The presented results on pectin content, the composition of the cell wall and the symplastic content of soluble carbohydrates strongly support the hypothesis that the changes in cell wall chemical properties may arise from restructuring of the already existing cell wall material (O'DONOGHUE and SOMERFIELD, 1998) instead of a new synthesis from stored carbohydrates. According to O'DONOGHUE and SOMERFIELD (1998) changes in cell wall structure are more a slowing down or final cessation of cell wall accumulation than a wall breakdown. This response „may reflect the immature nature of the spears after harvest". ZURERA et al. (2000) reported that cell wall thickness slowly but significantly increased in white asparagus spears stored at 20°C but not in those kept at 4°C. In terms of a cold-hardening response, the differentiated temperature dependence of changes in both stiffness and strength, and chemical cell wall properties may indicate a lack of elastic adaptation potential (HERPPICH et al., 2001a, b). It may also point out a prominent role of a continuation of maturation in a potentially fast growing plant shoot. The fact that the overall response is smaller at 20°C than at 10°C may result from a starvation effect at the higher temperature. At 20°C carbohydrate reserves necessary for growth may deplete much faster due to the significantly higher maintenance respiration (LIPTON, 1990).

A major produce quality-related short-term effect of cold hardening is osmotic adaptation (HERPPICH et al., 2001a, b). It is characterised by the increased cell sap concentrations of sugars, soluble proteins, amino acids and organic acids, which, in turn, diminishes the risk of cellular freezing by decreasing the actual freezing point of the cell content (SVENNING et al., 1997; STRAND et al., 1999). However, total osmotic content did not increase irrespective of the storage temperature used. In contrast, it seemed to slightly decline (approx. 3%) except when spears were stored at 0°C. In addition, soluble sugars, which contribute more than 35% to total osmotic content declined during storage. This may indicate that no reserve carbohydrates were available in the potentially fast growing spears. This is in accordance with the finding that no starch (own observation, PRESSMAN et al., 1989) or only very low starch content could be detected in spears (HSIAO et al., 1981). If starch is used as an intermediate store these results might indicate that it is depleted very fast after harvest as shown for carrots (STURM et al., 1999). Furthermore, despite the fact that fructans are the main storage polysaccharides in asparagus roots it has not been found in asparagus spears (PRESSMAN et al., 1993). Hence, in spears as in mature shoots exclusively free soluble sugars are the initial sources of both cell wall synthesis and respiration (PRESSMAN et al., 1989).

A relatively low sucrose content compared to fructose and glucose content as observed in our investigation has also been reported by others (e.g. HSIAO, 1981). In addition, it has been shown that at 20°C the sucrose content very rapidly, within 6 h, declines in the metabolically very active spear during the initial postharvest phase, while thereafter losses declined more gradual in freshly harvested spear to finally reach more or less constant low values (IRVING and HURST, 1993). On the other hand, the free reducing sugars declined either very slowly (glucose) or their contents were more or less constant (fructose) within a three days storage period. Reduction of respiration rapidly occurring in spear tips is assumed to represent carbohydrate starvation effects (IRVING and HURST, 1993).

In addition to glucose, fructose and sucrose no other soluble sugars have been detected in significant amounts in asparagus spears of all treatments. On the other hand, the cyclic sugar alcohol *myo*-inositol, not being measurable in fresh spears, accumulated at low but noteworthy amounts during storage. Although this *per se* occurred irrespective of the temperature regime, accumulation was lowest at room temperature. This may point out some relationship to the cold treatment. Indeed, it has been shown that this cyclitol is a central component of several biochemical pathways related to cold stress (NELSON et al., 1998; LOEWUS and MURTHY, 2000). However, it is also involved in plants' responses to salinity and drought stress. In this context, *myo*-inositol is the precursor of the important compatible solutes ononitol and pinitol (NELSON et al., 1998) and it is the initial step of the raffinose type polysaccharide biosynthetic pathway (AMIARD et al., 2003). On the other hand, *myo*-inositol is involved in the sugar interconvertion pathway that is necessary for the synthesis of cell wall matrix during secondary cell wall formation (MELLEROWICZ et al., 2001). Although *myo*-inositol may well play a multifunctional role in stored asparagus spears the stability of the water status largely reduces the probability of a direct a drought response. The interesting aspects of the induction of *myo*-inositol accumulation in asparagus certainly demand further experimentation.

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