Europ.J.Hort.Sci., **68** (2), 59–62, 2003, ISSN 1611–4426. © Verlag Eugen Ulmer GmbH & Co. Stuttgart

Effect of Low Oxygen Storage on Carbohydrate Changes in Onion (*Allium cepa* var. *cepa*) Bulbs

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Summary

Onion (*Allium cepa* var. *cepa*; cv. 'Sherpa', type Rijnsburger) bulbs were stored for 36 weeks at 2 °C under different oxygen concentrations (0.5, 1.0 and 21 %). Samples were collected after 9, 18, 27 and 36 weeks of storage. Water-soluble carbohydrates, respectively fructans were analysed using anion exchange chromatography combined with pulsed amperometric detection. Concentrations of total water-soluble carbohydrates in onion bulbs were different only for sampling dates after 27 and 36 weeks of storage. The reduction in water-soluble carbohydrates was highest during storage at 21 % oxygen and lowest during storage at 0.5 % oxygen. The reason for this effect was a reduced breakdown of fructans during low oxygen storage indicated by the fructan concentration and the degree of polymerisation. Our experiments' results suggest that vegetable crops containing higher concentrations of fructans are principally suitable for low oxygen storage.

Zusammenfassung

Einfluss einer Lageratmosphäre mit reduziertem Sauerstoffgehalt auf Veränderungen des Kohlenhydratgehalts bei Speisezwiebel (*Allium cepa* var. *cepa*). Speisezwiebel (*Allium cepa* var. *cepa*; Sorte 'Sherpa', Typ Rijnsburger) wurde über einen Zeitraum von 36 Wochen bei 2 °C und unterschiedlichen Sauerstoffkonzentrationen (0,5, 1,0 und 21 %) gelagert. Die Probennahme erfolgte nach 9-, 18-, 27- und 36-wöchiger Lagerung. Wasserlösliche Kohlenhydrate, insbesondere Fruktane, wurden mittels Anionen-Austausch-Chromatographie und gepulster amperometrischer Detektion analysiert. Unterschiede in den Konzentrationen der wasserlöslichen Kohlenhydrate in den Bulben der Speisezwiebeln konnten erst nach einer Lagerdauer von 27 und 36 Wochen nachgewiesen werden. Die Abnahme der wasserlöslichen Kohlenhydrate während der Lagerung war bei einer Lageratmosphäre mit 21 % Sauerstoff am höchsten und bei einer Lageratmosphäre mit 0,5 % Sauerstoff am geringsten. Der Ursache dieses Effekts lag im verringerten Fruktanabbau bei der Lagerung unter reduzierter Sauerstoffkonzentration, was sich in höheren Fruktankonzentrationen und Polymerisierungsgraden der Fruktane manifestierte. Die Ergebnisse unserer Versuche legen den Schluss nahe, dass Gemüsearten mit entsprechend hohen Fruktankonzentrationen grundsätzlich für die Lagerung bei niedrigen Sauerstoffkonzentrationen geeignet sind.

Introduction

Storability of onions is extended in low oxygen concentrations due to inhibition of sprouting, less neck rot and reduced weight loss (ADAMICKI 1997; SITTON et al. 1997). Reasons for quality maintenance effects of fruits and vegetables in low oxygen atmosphere are reduced respiration intensity and inhibited ethylene synthesis and action (BEAUDRY 1999). Reduced respiration under hypoxic conditions has been attributed to the restriction of a mitochondrial terminal oxidase (CHEVILLOTTE 1973), to the restriction of other oxidases with smaller affinity to O_2 than the cytochrome oxidase (BURTON 1982) or a restriction of initial steps of glucose oxidation (SOLOMOS 1997). Tolerance to hypoxia is influenced by several factors, for instance if commodities are climacteric or non climacteric, diffusion properties of stored plant organs or the stage of maturity (GRAN and BEAUDRY 1993; SOLOMOS 1997).

So far the problem of glycolysis has dominated studies of oxygen shortage research (e.g. KENNEDY et al. 1992; PERATA and ALPI 1993; CRAWFORD and BRÄNDLE 1996). Fructans are considered to play a role in tolerance to oxygen deficiency. Investigations on different species demonstrated, that long-term oxygen shortage caused an accumulation of soluble carbohydrates – especially fructans – in shoots and roots (ALBRECHT et al. 1993, 1997).

The common carbohydrate reserves of *Allium* species include glucose, fructose and sucrose, in addition to fructan oligosaccharides (DARBYSHIRE and STEER 1990). Fructan series with exclusively (2–>1) fructosyl-fructose linkages are present in *Allium cepa* L., i.e. neokestose-based series with chain elongation only at the glucose end of the original sucrose molecule and with chain elongation from both sides of the sucrose molecule, an inulin series and an inulo-*n*-ose series without terminal glucose (ERNST et al. 1998). Starch is far less abundant than the other carbohydrates (ERNST and BUFLER 1994; ERNST et al. 1999).

The effect of fructans in human nutrition has become more and more important in recent years, thus challenging research activities on fructan metabolism in plants. Vegetable crops like onion, leek, asparagus, and sprouts from cereals contain rather high amounts of fructans (ERNST and FELDHEIM 2000).

The experiment described here was conducted to give evidence of the changes in water-soluble carbohydrates, respectively fructans in onion bulbs exposed to low oxygen concentrations (1.0 % O_2, 0.5 % O_2) during cold storage.

Materials and Methods

Plant material and storage conditions

In September 1999 cured onions (cv. 'Sherpa', type Rijnsburger) from commercial field production were furnished from south east Bavaria and kept under CA-conditions at 2 °C immediately after transport. Oxygen concentrations in the storage containers were 0.5, 1.0 and 21 %, the CO_2-concentration for all treatments was below 0.3 %. Gas concentrations were measured and regulated continuously using a paramagnetic analyser for O_2, an infrared analyser for CO_2. Humidity was higher in containers with 21 % O_2 (85–89 %) than in the storage containers with low O_2-concentrations (78 and 82 %). At intervals of 9 weeks (16.11.1999, 18.1.2000, 23.3.2000, 23.5.2000) 16 onion bulbs of each oxygen level were taken out of the storage containers to determine carbohydrate fractions separately for each bulb.

Carbohydrate extraction and analysis

Carbohydrates were prepared for separation on anion exchange chromatography (AEC) by extracting 60 mg of dried tissue twice for 15 min in 7 ml boiling water saturated with calcium hydroxide. The two extracts were centrifuged, and the combined supernatant (14 ml) was passed through a 0.22 µm filter. The chromatographic profiles of fructans were established using AEC combined with pulsed amperometric detection (PAD) on a Dionex® Series 300DX (Dionex Corp., Sunnyvale, CA, USA) ion chromatograph fitted with a CarboPac® PA-1 column (CHATTERTON et al. 1989; SHIOMI et al. 1991). Flow rate was 1 ml min^{-1}. Carbohydrate elution was effected under alkaline conditions (150 mM NaOH). The high pH (12–13) of the eluent converts hydroxyl groups of the oligosaccharides into oxyanions. The degree of oxyanion interaction with the anion exchange resin determines carbohydrate retention times. Adding a competing ion such as acetate (0–500 mM NaOAc) to the eluent reduces the retention times.

Part of the extract was hydrolysed with HCl for determination of total glucose and total fructose. Total water-soluble carbohydrates (WSC) were calculated by summing up the amount of glucose and fructose after hydrolysis. Total fructan was calculated by subtracting free glucose, fructose, and sucrose from WSC. The calculated fructose/glucose ratio was used as an indicator for the average degree of polymerization (DP) of fructans, considering that each fructan molecule contains one glucose monomer.

Statistical analysis

The analysis of variance was conducted with statistic software SAS 6.12 (SAS Institute Inc., Cary, NC, USA) using the ANOVA procedure and Tukey's studentized range test.

Results

The dry matter concentration of the onion bulbs was at a level of 12–13 % during the whole experiment of 36 weeks of storage. No significant differences in dry matter concentrations for sampling dates and oxygen concentrations were detected.

Changes in water-soluble carbohydrates were not evident for the first two sampling dates (9–18 weeks of storage). The last two sampling dates (27–36 weeks of storage) gave a typical pattern with the lowest level of total water-soluble carbohydrates for the control treatment (21 % O_2) and the highest level of total water-soluble carbohydrates for the treatment '0.5 % O_2' (Fig. 1).

Mono- and disaccharides were the dominating carbohydrate fraction of the stored onion bulbs (cv. 'Sherpa') with a concentration of up to 480 mg g^{-1} dry matter (Fig. 1). Changes in concentrations of glucose and fructose during storage didn't show a clear pattern in time course and for the different oxygen levels of storage atmosphere. Sucrose concentration was significantly higher for low oxygen levels during storage compared with bulbs of the control treatment.

Fructans were present as neokestose-based series and inulin series while the inulo-*n*-ose series was present only in trace amounts, except for the control treatment (21 % O_2) of the last two sampling dates inulo-*bi*-ose (F_2) was increased significantly (Fig. 2). The fructan concentration was significantly reduced only for the last sampling date after 36 weeks of storage. Differences of fructan concentrations between different oxygen levels during storage were significantly higher for '0.5 % O_2' than '1.0 % O_2' and lowest for 'control' (21 % O_2). The average degree of polymerization – calculated by the fructose/glucose ratio after fructan hydrolysis – was lowest for the control (ranging from 3.2 to 3.9) for all sampling dates and highest at '0.5 % O_2' level (ranging from 3.8 to 6.7). The chromatograms of Fig. 2 confirm that the degree of polymerization of fructans after cold storage is higher for onion bulbs stored at low oxygen levels compared to the control treatment.

Discussion

Effects of ultra-low-oxygen and controlled-atmosphere storage have been considered mainly under the aspect of the product's saleability after storage using the product's colour, texture, and flavour of the product as criteria (WEICHMANN 1986). In our experiment colour, texture, and flavour of stored onion bulbs were not affected negatively by low oxygen concentrations (data not shown). Sprouting, monitored by determination of leaf ratio (for method see: ERNST et al. 1999), was reduced in onion bulbs stored at low oxygen levels compared to the control (21 % O_2) (data not shown). That means, during low oxygen storage there weren't observed any negative effects, concerning the above mentioned parameters.

It is known that decomposition of carbohydrates, especially sucrose and starch is slowed or inhibited by low oxygen levels during cold storage, a review for a number of fruits and vegetables was given by WEICHMANN (1986). Many studies have investigated the relationship between fructan metabolism in higher plants and cold temperature (e.g. RUTHERFORD and WESTON 1968; POLLOCK 1986; POLLOCK and LLOYD 1987; PONTIS 1989). To the authors' knowledge, there is no information available concerning the fructan metabolism of fructan accumulating plant organs during low oxygen storage.

The onion bulbs (cv. 'Sherpa') used in this experiment showed the typical pattern of soluble carbohydrates, respectively fructans (ERNST et al. 1998) with a relatively high percentage of glucose, fructose, and sucrose. Low oxygen storage of onion bulbs inhibited su-

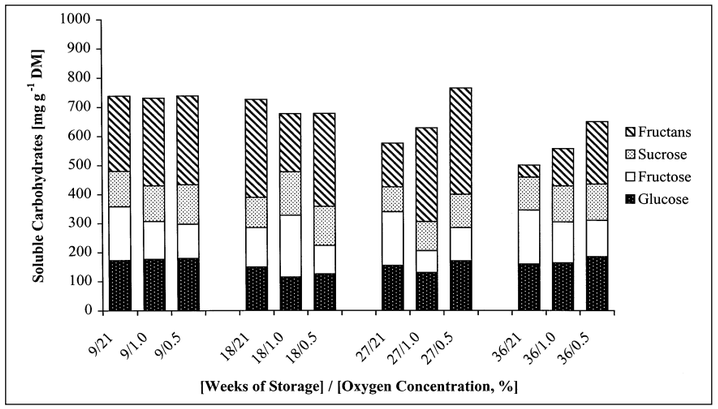


Fig. 1. Changes in water-soluble carbohydrate concentrations during storage at 2 °C and different oxygen concentrations (statistical analysis was done separately for each storage period ($P \leq 0.05$)).

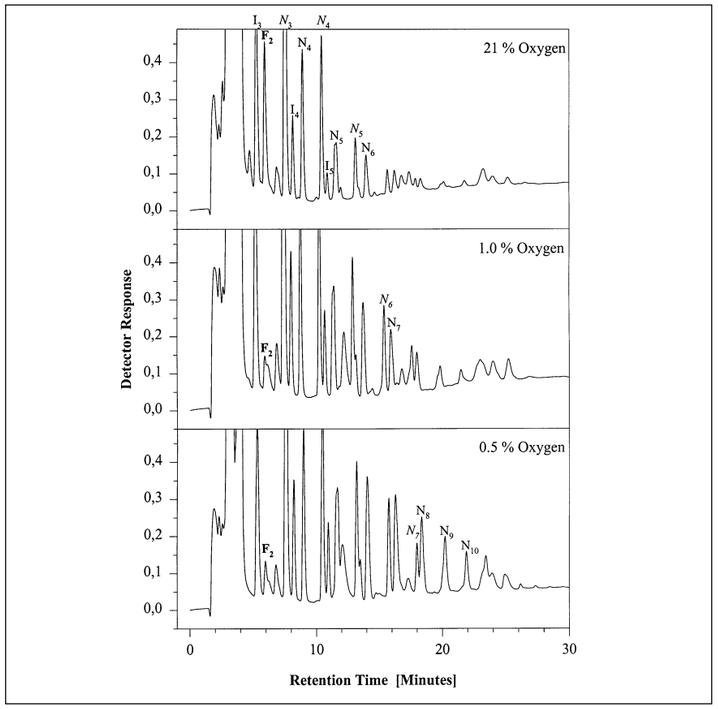


Fig. 2. Chromatograms of water-soluble carbohydrates from onion bulbs after 36 weeks of storage at 2 °C and different oxygen concentrations. I = inulin series, N = neokestose series, *N* = neokestose series with chain elongation only at the glucose side of sucrose and F = inulo-*n*-ose without terminal glucose; the subscript indicates the degree of polymerization.

crose loss and slowed fructan-breakdown significantly, indicated by the degree of polymerization of fructans and the relative absence of inulo-*bi*-ose, which can be regarded as an indicator for fructan breakdown (ERNST et al. 1996). Increasing or non-decreasing concentrations of glucose, fructose and also of sucrose – especially for the control treatment (21% O_2) – might be caused by fructan breakdown.

There are no doubts about the occurrence of alcoholic fermentation in plant tissues under anoxia, but in contrast to an accelerated fermentation many plants accumulate sugars and also fructans at low oxygen concentrations (ALBRECHT et al. 1997). Measurement of oxygen in bulbs of onions during storage at ultra-low-oxygen conditions showed oxygen deficiency in bulb tissue, however there was no damage observed caused by anaerobic respiration (PRAEGER 2001). Therefore one might conclude that vegetable crops with fructan metabolism are principally suitable for low oxygen storage, which is supported by the results of our experiments and the data from other publications (e.g. ALBRECHT et al. 1997). Further investigations will better define the storability of other vegetable crops with fructans as reserve carbohydrates under low oxygen conditions.

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Received December 10, 2002 / Accepted January 27, 2003

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