Ethanol content in cut roses at low oxygen atmosphere storage

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ABSTRACT: Low oxygen atmosphere is beneficial for preservation of fresh cut roses. Flower tolerance to specific atmosphere yielding anaerobic products was determined. A suitable gas mixture of ULO conditions (0.8% oxygen and 0.1% carbon dioxide) was shown to lead to elevated ethanol production in tissues. The tissue ethanol content in ULO and RA atmosphere (21% oxygen and 0.03% carbon dioxide) was 300 mg/l and 6 mg/l, respectively. After the exposure to ULO conditions when the material was in air ventilation under cold storage, ethanol decreased to the trace concentration comparable with that at the beginning of storage. The oxygen stress caused only small fluctuations in the content of acetaldehyde with difference from 3 to 6 mg/l. The presence of acetaldehyde in the pulp of cut roses indicated a negligible degree of injury by low oxygen. There were only minimal differences between ULO and RA in non-volatile compounds such as sucrose, glucose and fructose. Buds of cut roses did not open during the storage in ULO conditions and in the prolonged phase of air ventilation their opening was delayed for the next 15 days of cold storage.

Keywords: *Rosa hybrida* L.; ethanol; acetaldehyde; low oxygen; content of sugar

Physiological and biochemical factors associated with tolerance of cut roses to low oxygen and high CO_2-treatments are not well understood.

One focus of research was preservation of quality during prolonged storage by sucrose (DEAMBROGIO et al. 1991; ICHIMURA et al. 1999) and floral preservatives containing antibacterial chemicals (HOOGERWERF, VAN-DOORN 1992; TORRE, FJELD 2001; PHAVAPHUTANON, KETSA 1990; RUTING 1991). A noticeable improvement of quality preservation and vase life prolongation was reported for ethylene antagonists (MOR et al. 1989). Also environmental conditions during cultivation can influence the postharvest life of cut roses (HALEVY, MAYAK 1979; URBAN et al. 1995).

Another focus was the accumulation of acetaldehyde and ethanol in response to elevated CO_2 and lowest possible oxygen in the atmosphere.

The knowledge of the low oxygen limit is critical for optimising the gaseous storage of cut flowers. The optimum storage atmosphere is just above the lowest achievable aerobic metabolism still before the development of ethanol. Storing cut roses under low oxygen provided some benefit in improving the vase life (RIO et al. 1989). On the other hand, undesirable responses included, among others, the induction of fermentation, the development of disagreeable tissue injury of petals. SHELTON et al. (1997) suggested that the sequence of oxygen and carbon dioxide exposure affected floral tolerance to several controlled atmosphere treatments.

For the roses it is known that a sugar supply to a vase solution can increase the vase life, the most probable explanation being that sugar is used as a substrate for respiration, maintenance, synthesis and osmoregulation and thus delays senescence. However, MARISSEN (2001) claims that the saccharide concentrations in leaves and flower buds are not simple indicators of the subsequent vase life.

Differences in fermentation responses of cut roses to 0.8 kPa of oxygen and about 0.1 kPa of carbon dioxide atmosphere provide useful material to discuss various aspects of rose tissue tolerance to the gases. In this study, our objective was to study the changes in ethanol and acetaldehyde content in relation to saccharide substrate in stored cut roses treated with low oxygen atmosphere.

MATERIALS AND METHODS

FLOWER SUPPLY, INITIAL TREATMENT AND STORAGE

Single stem plants of *Rosa* × *hybrida* L. were grown in rock wool in a glass greenhouse in natural radiation inside the greenhouse. Cuttings were taken from the middle and lower position of stems harvested at a normal commercial stage of unopened flower development. The basal stem of the cutting was placed in a water solution and was held in that solution during the whole time of storage.

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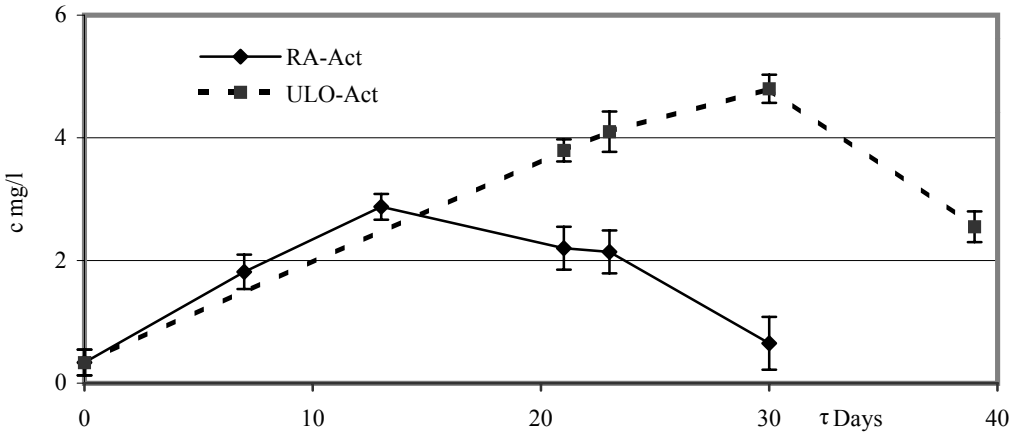

Fig. 1. Acetaldehyde concentrations in the tissue of cut roses after 37 and 30 days in ULO and RA storage, respectively. The phase in ULO lasted for 21 days, subsequently the cut roses were stored at a cold storage temperature and in normally composed air. Each value represents 3 replications and vertical bars indicate SE, $P < 0.05$

There were two levels of oxygen in our experiments that were conducted in 400 l gas-tight containers ULO (0.6–0.8% oxygen and 0.1% CO_2 – ultra low oxygen); storage in regular atmosphere – RA (21% oxygen and 0.03% carbon dioxide) took place at a storage room. To achieve the desired concentration of O_2 , flushing with N_2 was carried out. The storage temperature was 3°C. The concentrations of CO_2 and O_2 were continuously monitored by gas analysis (Arelco, ARC, France) connected to a process computer. Gas concentration was checked each hour. After 21 days the container was opened and the flower was transferred into the air atmosphere.

POSTHARVEST HANDLING AND MEASUREMENT

Before the postharvest life tests, cut roses of all variants were placed only in a cool room (3°C, 92–94% relative humidity) till the end of the experiments. Bending of pedicels, wilting (loss of turgor), blueing and discoloration of petals indicated the end of the vase life and was registered daily.

Ethanol and acetaldehyde measurements were performed individually on flowers upon their removal from containers. An additional measurement followed after the flowers had been held in air at 3°C when a single composite frozen sample was analysed. 1 ml sample of juice from the stem tissue was filtered and immediately frozen.

Acetaldehyde and ethanol in the frozen juice were analysed by GC (packed column with Porapak P). 1 µl of aqueous samples was injected into a sample block fitted with Teflon, an inert material. Four peaks from the chromatogram were evaluated, two of them were quantified by external standard of these compounds (acetaldehyde, ethanol) and expressed in mg/l for each compound.

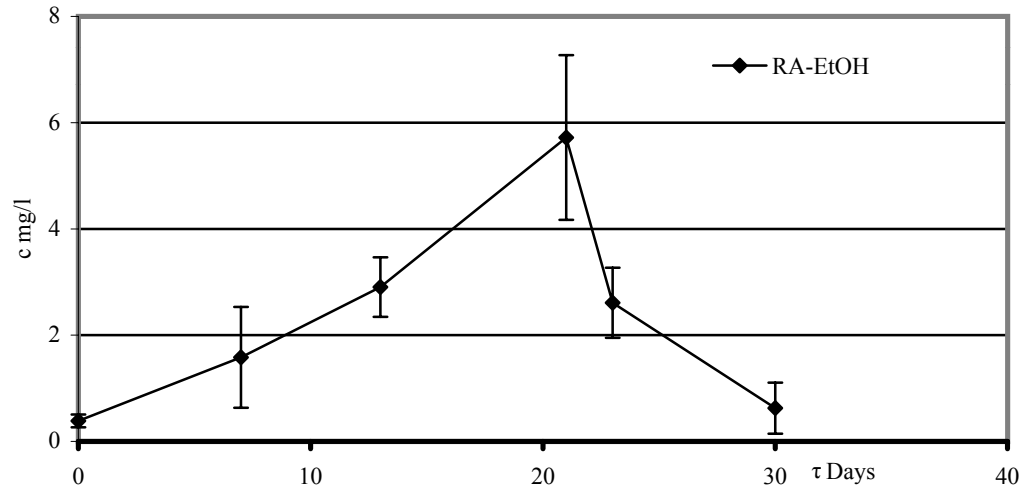

Fig. 2. Ethanol concentrations in the tissue of cut roses in regular atmosphere (RA). Each point is the mean of 3 replications and vertical bars indicate SE, $P < 0.05$

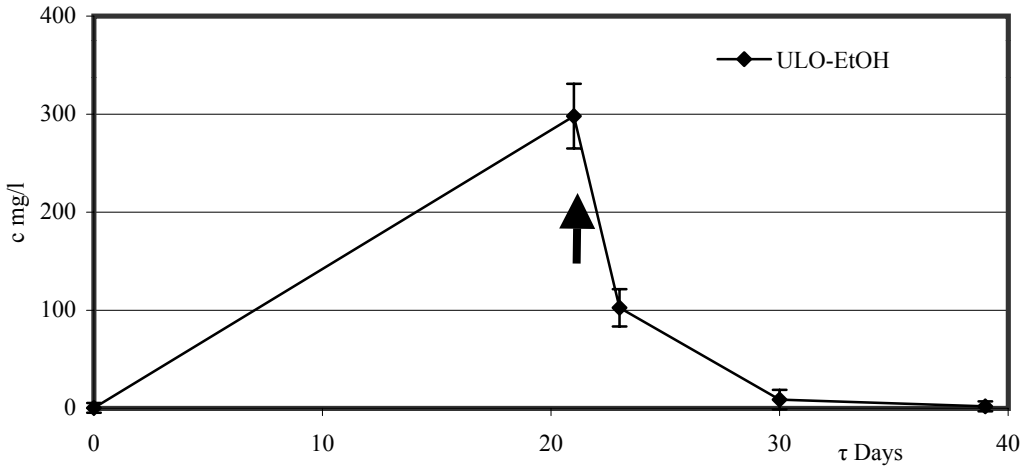

Fig. 3. Ethanol concentrations in the tissue of cut roses exposed to ultra low oxygen content (0.6–0.8%) and 0.1% carbon dioxide. Each point is the mean of three replications and vertical bars indicate SE, $P < 0.05$

SUGAR ANALYSIS BY HPLC

From homogenised frozen juice, sugars (sucrose, glucose, fructose) were analysed by HPLC by isocratic elution with water as a mobile phase, on the column (Polymer IEX H^+ form, 250 × 8 mm, Watrex) with 50°C temperature of column and flow rate 0.7 ml/min with refractometric index detection. Each sugar was expressed via external standard in mg/l.

RESULTS AND DISCUSSION

OCCURRENCE AND POTENTIAL EFFECTS OF ANAEROBIC METABOLITES

Alcohols are produced in plant tissues in several ways. Ethanol production is primarily associated with glycolytic activity in tissues, which is enhanced by anaerobic conditions. The effect of high CO_2 atmosphere was not observed. Low oxygen injury, very well-known from apple fruits, was reported to be associated with ethanol accumulation more than 2,500 µl/l in Delicious apple tissue (NICHOLS, PATTERSON 1987), which suggests that the injury is post-anoxic in nature. Anaerobic metabolites are often considered to be the cause of storage disorders with which they are often associated. Many metabolic studies of the survival of plant tissue in the absence of oxygen are focused on possible toxicity of the main glycolytic end products. Plants from the extreme atmosphere are differentiated only by the concentration of ethanol that is always higher in ULO than in the variant of regular atmosphere (RA) where it is fifty times lower than in ULO storage (Figs. 1 and 2). The higher ethanol production is in the tissues that are kept permanently under anaerobic conditions (0.6–0.8% O_2). The juice from such fruits is a result of the fast an-

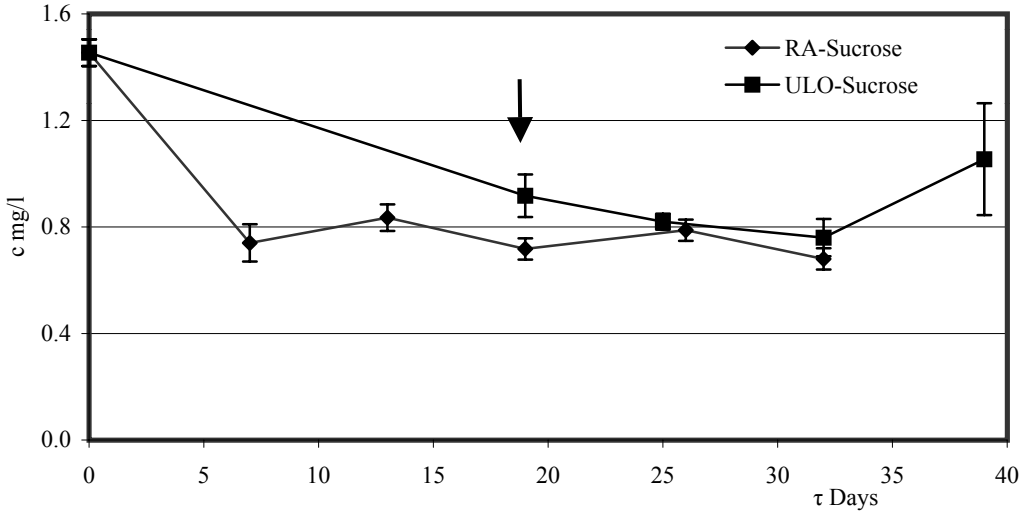

Fig. 4. Effect of exposure to ULO and RA conditions on sucrose content in the tissue of cut roses. Vertical bars represent standard error of the mean from three replications, $P < 0.05$. The arrow indicates the date of transfer to air

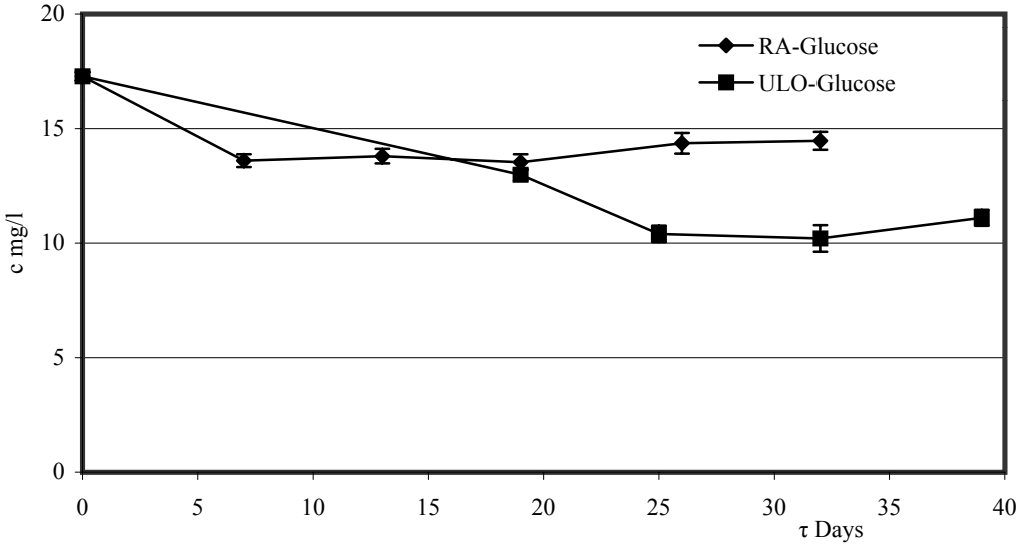

Fig. 5. Effect of exposure to ULO and RA conditions on glucose content in the tissue of cut roses. Vertical bars represent standard error of the mean from three replications, $P < 0.05$. The arrow indicates the date of transfer to air

aerobic glycolytic process. When the flowers were transferred to the air (after 21 days), the concentration of ethanol in the tissue decreased exponentially (Fig. 2). At the temperature of cold storage, this degradation phase took the same time as the period in low oxygen.

The differences in the content of acetaldehyde are not statistically significant. Traces of acetaldehyde are seen during the growing phase, the concentration not surpassing 0.5 mg/l. The response of flowers on the cutting is similar: the accumulation was practically identical but it differs in the nominal value (Fig. 3). It is only during the air ventilation phase (after 21 days, Fig. 3) that the concentration of acetaldehyde lowers to half values.

Exogenously applied ethanol (SALTVEIT, MENCARELLI 1988) or endogenously synthesised ethanol (KELLY, SALTVEIT 1988) inhibited the ripening of whole tomato fruits at various maturity stages. Ethanol also delayed the senescence of carnation flowers (SATLER, THIMANN 1980). Cut roses are not in the category of climacteric fruit as the tomato fruit and apple fruit but the response to low oxygen is similar.

The level of sugars will be important if it is limiting vase life and bud opening. The application of sugars to the vase solution in order to increase the vase life is a common practise. MARISSEN (2001) denied any relation between vase life and sugar pool in leaves or flower buds. In Figs. 4 to 6 one can see that the concentration of glucose and fructose declined during the ULO storage and following aeration phase. Whereas the largest change in the content was in fructose, a relatively smaller decrease was measured in the content of glucose.

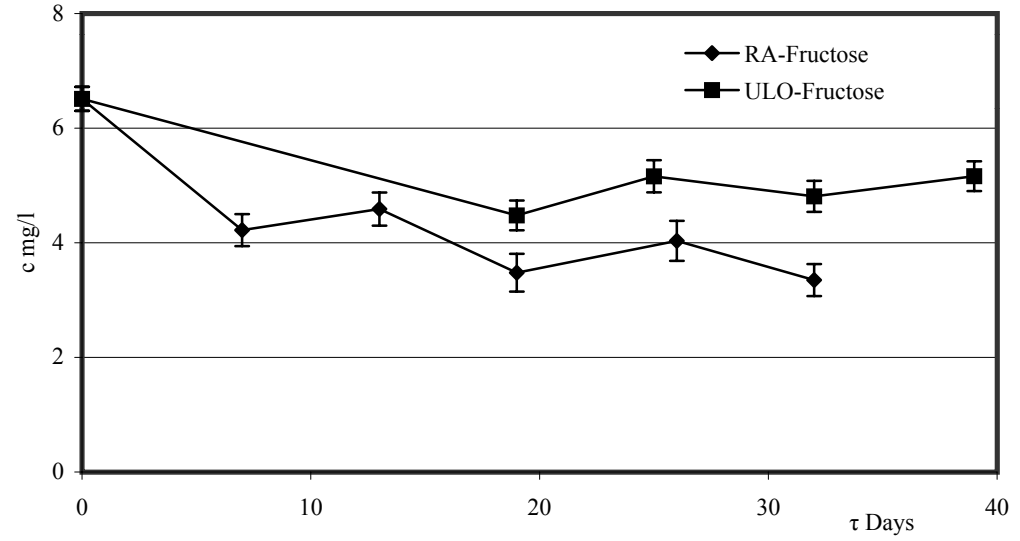

Fig. 6. Effect of exposure to ULO and RA conditions on fructose content in the tissue of cut roses. Vertical bars represent standard error of the mean from three replications, $P < 0.05$. The arrow indicates the date of transfer to air

POSTHARVEST LIFE AND QUALITY EVALUATION

The loss of ornamental value measured as wilting symptoms, chlorotic and necrosis areas on the leaves, discoloration of petals was not observed in flowers in low oxygen conditions. However, buds of cut roses did not open during the storage in ULO conditions and in the subsequent prolonged phase of air ventilation their opening was delayed for the next 15 days of cold storage.

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Obsah etanolu v pletivu růží během uložení v prostředí s nízkým obsahem kyslíku

ABSTRAKT: Atmosféra s nízkým obsahem kyslíku je prospěšná pro udržení svěžesti řezaných růží. Byla stanovena tolerance ke specifické atmosféře, v níž byly stanoveny produkty anaerobiózy. Podmínky plynné směsi v ULO (0,8 % kyslíku a 0,1 % oxidu uhličitého) ukázaly na zvyšující se produkci etanolu v pletivu. Obsah etanolu v pletivu byl v ULO atmosféře 300 mg/l a s normálním obsahem kyslíku (21 % kyslíku a 0,03 % oxidu uhličitého) byl 6 mg/l. Obsah etanolu klesal na zbytkové hodnoty, jakmile se řezané růže vystavily do vzduchu v chladírenském skladování. Kyslíkový stres způsobil jen malé rozdíly v obsahu acetaldehydu, který kolísal mezi 3–6 mg/l. Rozdíly v obsahu acetaldehydu ve šťávě z řezaných růží ukazovaly jen na malé poškození v atmosféře s nízkým obsahem kyslíku. Obsah netěkavých složek, jako je sacharóza, glukóza a fruktóza, v ULO a v atmosféře s normálním obsahem kyslíku trvale klesal. Poupata řezaných růží během uložení v ULO se neotevřela a ve fázi aerace v následujících 15 dnech uložení na vzduchu bylo toto otevírání zpomaleno.

Klíčová slova: *Rosa hybrida* L.; etanol; acetaldehyd; atmosféra s nízkým obsahem kyslíku; obsah cukru

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