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Formation of anaerobic products in sweet cherries and their partial release into ambient atmosphere under low oxygen storage

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Summary

Concentrations of acetaldehyde, ethanol and ethyl acetate in the flesh of ripe, sweet cherries were consistently enhanced by very low oxygen conditions in the ambient atmosphere. At the onset of storage the initial concentration of ethanol in the flesh was 6.0 and 6.4 mg l^{-1} for the cvs. 'Kordia' and 'Vanda' respectively. After 28 days under anaerobic conditions the concentration in the flesh of cv. 'Kordia' was 5,502 ± 35 mg l^{-1}, which during the next 10 days of storage in air was increased to 6,476 ± 771 mg l^{-1}. The production of ethanol under anaerobic conditions increased during the storage period from 101 µg $kg^{-1}h^{-1}$ to 550 µg $kg^{-1}h^{-1}$ from the 16th to the 27th day. The ethanol released from fruit stored at ULO and CA atmospheres was accumulated in the ambient atmosphere at very low concentrations, two orders lower than in the anaerobic atmosphere, at concentrations from 1.2 to 1.6 µg $kg^{-1}h^{-1}$. The concentration of ethyl acetate during the first 10 days under very low oxygen atmosphere did not increase, because biosynthesis practically ceased. At the start it was 0.76 mg l^{-1} and after 10 days it had increased to only 0.88 mg l^{-1}. Until the fruit is exposed to a normal supply of oxygen from the air, there is no change in the esterification rate. Some quantitative relations relate to the production and subsequent release of ethyl acetate from intact fruit, which under subsequent ambient atmosphere storage was detected at very low trace concentrations, of 20 to 80 nl l^{-1} overall, for all the different storage regimes.

Introduction

The analysis of gas compounds emitted by stored fruit is an interesting research area for characterizing different varieties of the same fruit or for checking its condition by monitoring the volatiles in the ambient atmosphere. A knowledge of headspace modification can help to identify changes in the headspace profile induced by stress. Modification of the gas mixture, a general stress indicator is a response which has already been intensively investigated (Tiang et al., 2001; Spotts et al., 2002; Martines-Romero et al., 2006). The emitted compounds, namely acetaldehyde, ethanol and ethyl acetate, are formed by the enzymatic conversion of glucose by anaerobic glycolysis. Apart from these compounds, a wide range of chemically different compounds, some of them of approximately very high polarity, can be identified depending on the particular sampling technique employed. A commonly used method is the "dynamic headspace" method. The volatiles are concentrated onto an adsorbent, such as activated charcoal carbon molecular sieves or porous polymers, e.g. Tenax (Vercammen et al., 2000) followed by liquid or thermal desorption. Presently, thermal desorption is the method of choice because it can be coupled on-line to the analytical system, which guarantees the highest sensitivity and reduces the risks of contamination. For thermal desorption, the adsorbents have a number of disadvantages, the most important of which are that high molecular weight and polar solutes can only be desorbed at relatively high temperatures.

Very low oxygen conditions are those where the oxygen concentration in the atmosphere surrounding tissues is insufficient to support aerobic metabolism. Such conditions induce anaerobic respiration, when acetaldehyde and ethanol accumulate. Brief periods of anaerobic conditions reduce post-harvest decay, help maintain general fruit quality, and are potential disinfestation treatments. Low-O_2 conditions are considered beneficial for apple storage, as they maintain colour and firmness longer than when stored in air (Dixon and Hewett, 2000; Romanazzi et al., 2003). Postharvest loss of quality of sweet cherries is essentially due to softening and loss of acidity, and microbiological decay also influences the storability of sweet cherries (Petracek et al., 2002; Esti et al., 2002; Bernalte et al., 1999; Lurie, 1998; Venturi et al., 2002).

The objective of this study was to monitor the production of the anaerobic metabolites ethanol, acetaldehyde and ethyl acetate under the experimental gas mixtures of ultra-low oxygen (ULO), controlled atmosphere (CA) and very low, fluctuating anaerobiosis (FAN) with 0.2 - 0.9 % of oxygen, in sweet cherry fruits stored at a temperature of 3°- 3.5°C. After identifying the gaseous compounds released from intact fruit into the surrounding atmosphere, the concentrations were measured.

Material and methods

Plant material and preparation of atmosphere

Sweet cherry fruits were purchased from Agro Stošíkovice in South Moravia. Cv. 'Vanda' and 'Kordia' were harvested at the optimal stage of maturity (based on visual appearance) and after harvest were chilled to 2.5 - 3.0°C for 6 hours. The fruits were divided into four groups, treated as follows:

FAN - fluctuating anaerobiosis - 0.2 % O_2 + 0.9 % CO_2,
CA - controlled atmosphere - 2.0-2.5 % O_2 + 6.5-7.0 % CO_2
ULO - ultra-low oxygen - 1.0-1.2 % O_2 + 0.5 % CO_2
RA - regular atmosphere - 21 % O_2 + 0,03 % CO_2

These gas mixtures were maintained for 28 - 31 days. The chamber with FAN atmosphere was fitted with an additional circuit for sampling the ambient atmosphere, which allowed the sampling of fruit for measuring of metabolit volatiles at three terms as shown in Fig. 1-3. The CO_2 and O_2 levels in the chambers were monitored with a precision of 0.1 % (Dual gas analyser O_2/CO_2, model TS12E, Fa Arelco ARC, Fontanay, France) every few minutes initially and once a day thereafter. A concentration of <0.5 % O_2 was reached within ten minutes of commencing purging and remained constant until the chambers were opened again.

Collection of samples and measurement of substances in the liquid phase

At the beginning and at the end of the gas mixture treatments, a homogenous juice was separated from 12 fruits of each cultivar for each treatment and frozen to -18°C. Chromatographic analysis followed. The defrosted samples were filtered with Glass Pre-Filters, Polypropylene Housing, 0.2 µm Pore Size, (Alltech, Associates, Inc., IL) and 1 µl of the non-diluted filtrate was injected into the packing

column (length 1.2 m, inner diameter 3 mm) filled with Porapak P (Watters, Ass., Inc., Framingham, Mass, USA). Crushed teflon was added periodically to the injection space of the chromatograph to adsorb the ballast substance contained in the liquid sample. GC conditions: oven temperature 92°C, detector temperature 150°C, injector temperature 120°C, carrier gas He (12 ml/min), FID, Chrom 5, Laboratory Equipment, Czech Republic. The quantitative study of acetaldehyde, ethanol and ethyl acetate was carried out with absolute calibration. The calibration and the analyses were carried out under identical conditions.

Dynamic sampling

The fruits was placed into spherical 500 ml jars. The jars were sealed with two gas ports. Purified air flowed into the jars at 50 ml min^{-1} at 20°C prior to sampling. To the outlet port was sealed the enrichment column filled by Tenax TA. After the 180 g of sweet cherry fruit had equilibrated in the sampling assembly for 1 h, dynamic sampling was performed by connecting a packed enrichment tube to the outlet of the sampling unit and applying a flow of air at 50 ml min^{-1} for 60 min. Conditioning of the enrichment column packed with Tenax TA was performed by heating at 300°C for 0.3 h under a flow of 100 ml min^{-1} of nitrogen. Desorption of the trapped components was carried out using a TD-2 thermal desorption unit mounted on top of the GC Agilent 4890D injector. For all experiments desorption was in the splitless mode using helium at a flow rate of 150 ml min^{-1}. The TD-2 was programmed from 190°C to 200°C with a final time of 2 min. When desorption was completed, the split valve was closed after 0.7 min.

The capillary GC-TD system consisted of an HP 4890D GC. The column used during all experiments was a 30 m 0.25 mm I.D and 0.25 μm film. The oven was programmed from 35°C to 200°C at a rate of 3°C min^{-1}. Helium was the carrier gas at 1.2 ml min^{-1}. The instrument was equipped with a thermal desorption (TD-2) unit for dynamic sampling.

Statistical analysis

Comparisons were made of different storage conditions, cultivars by using Unistat, versin 7 (by means of the standard error method) and quantification of content during the gas treatment and subsequent storage in a normal atmosphere at 3°C. Differences of mean values were judged using a significance level of p=0.05.

Results and discussion

Content of metabolites under different storage conditions

At the onset of storage the initial concentration of acetaldehyde in the flesh was 4.6 and 4.2 mg l^{-1} for the cvs. 'Kordia' and 'Vanda' respectively. At the same time onset of storage in healthy fruits there was ethanol content 15.1 and 6.4 mg l^{-1} respectively for these same cultivars (Fig. 1 and 2). The concentration of ethyl acetate in the first 10 days under a very low oxygen atmosphere did not increase by much, because formation of this metabolites were stopped. At the start there was 0.76 mg l^{-1} and after 10 days were increased to only 0.88 mg l^{-1} (Fig. 3). Not until the fruit is exposed to a full supply of oxygen in air is there a change in the rate of ethyl acetate (Fig. 3). After the end of the storage periods and the influence of the gas mixtures, significant differences in ethanol, acetaldehyde and ethyl acetate in the fruit flesh were seen (Fig. 1-3). The highest ethanol concentration was observed after 30 - 35 days under FAN conditions, being 7.0 g/l in the flesh of the fruit (Fig. 2). For at least 10 days after being returned to aerobic conditions, the ethanol that was produced during the anaerobic phase had still not degraded. The gas mixtures ULO and CA did not promote the accumulation of anaerobic metabolites such as acetaldehyde and ethanol (Fig. 4 and 5), and both the cultivars demonstrated tolerance of anaerobiosis. The higher concentration of these alcohols and esters with short C-chains suggests fermentative metabolism and increased esterase activity (Mattheis et al., 1992, Meheriuk et al., 1995).

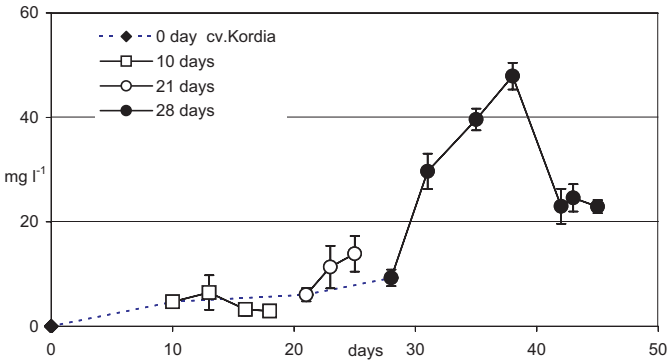

Fig. 1: Concentration of acetaldehyde in flesh of fruit stored in FAN – fluctuating anaerobiosis – 0.2 % O_2 + 0.9 % CO_2, after 10, 21 and 28 days exposed to air, (means and standard errors, n = 6).

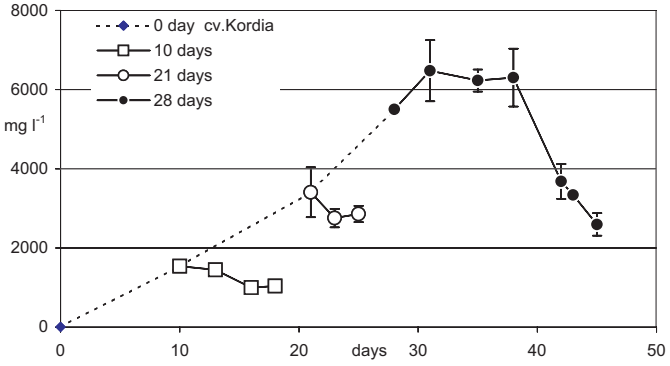

Fig. 2: Concentration of ethanol in flesh of fruit stored in FAN – fluctuating anaerobiosis – 0.2 % O_2 + 0.9 % CO_2, after 10, 21 and 28 days exposed to air (means and standard errors, n = 6).

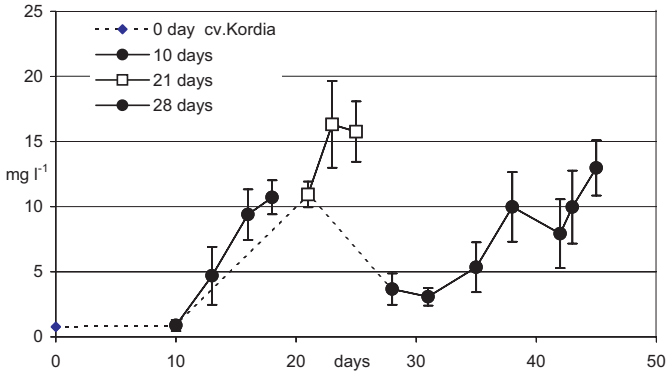

Fig. 3: Concentration of ethyl acetate in flesh of fruit stored in FAN – fluctuating anaerobiosis – 0.2 % O_2 + 0.9 % CO_2, after 10, 21 and 28 days fruit were exposed in air (means and standard errors, n = 6).

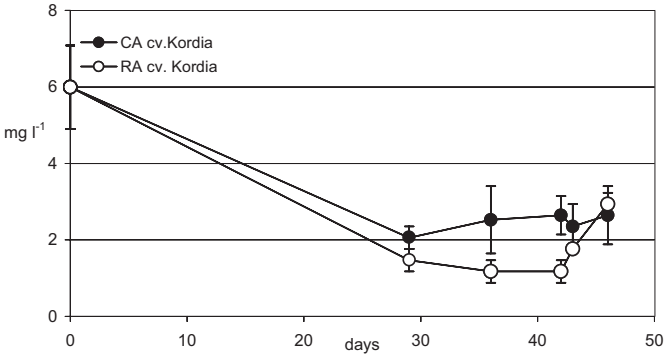

Fig. 4: Effect of O_2 level on the ethanol (EtOH) in pulp of cvs. 'Kordia' and 'Vanda' in ULO – ultra-low oxygen – 1.0-1.2 % O_2 + 0.5 % CO_2 (means and standard errors, n = 6).

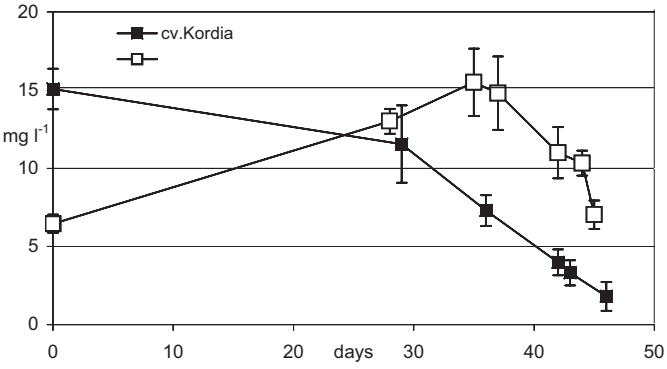

Fig. 5: Effect of O_2 level on the ethanol (EtOH) in pulp of cvs. 'Kordia' and 'Vanda' in CA – controlled atmosphere – 2.0-2.5 % O_2 + 6.5-7.0 % CO_2 (means and standard errors, n = 6).

The change in acetaldehyde, ethanol and ethyl acetate concentration after removal of fruit from low O_2 atmosphere

Removal of fruit from a stress atmosphere of 0.2 - 0.9 % O_2 FAN and transfer to an atmosphere of 21 % O_2 resulted in a moderately exponential decline in EtOH (Fig. 2). The time taken for acetaldehyde and EtOH levels to return to those before the introduction of stress is dependent on the time duration of the stress. After the second removal from anaerobic conditions (after 20 days), the EtOH levels did not significantly decline. However, after 30 days the ethanol production always increased, which confirmed the much reduced capacity of the fruit flesh to oxidize the accumulated compounds into their simple components. Probably a period of around 30 days under very low O_2 concentrations represents the maximum period for the fruit to be able to metabolize the ethanol before physiological failure state in (Fig. 1, 2 and 3).

Formation of ethyl acetate

A brief period of aerobic conditions enhances concentrations of ethyl acetate and anaerobic volatiles while suppressing acetate esters and acetaldehyde for a wide range of apple cultivars (DIXON and HEWETT, 2000). Oxygen is considered to be an essential co-factor for esterification of alcohols in fruit tissue by supplying NADH from aerobic respiration. In the absence of O_2, esterification reactions stop and concentrations of free alcohol increase. On return to aerobic conditions, these alcohols are metabolised either to esters or to shorter-chain compounds before esterification or they diffuse out from the tissue. The rate of release of gases from the intact skin can be formulated as a permeability constant (k - μg $kg^{-1}h^{-1}$) related to the concentration of compounds in the percolating gas flowing over the intact fruit to the concentration of these compounds occuring in the liquid phase in the fruit flesh. In the fruit from anaerobic conditions the permeability constant k value at 10 days was established as 1.28 10^{-4} μg $kg^{-1}h^{-1}$, at 20 days as 0.98 10^{-4} μg $kg^{-1}h^{-1}$, and at 30 days as 0.68 10^{-4} μg $kg^{-1}h^{-1}$. However, there are additional, undesirable chemicals produced which can also affect the quality of fruit. Ethyl acetate production is linked to off-flavours that are persistent after CA storage for 7 days or more, for many kinds of fruit (LARSEN and WATKINS, 1995; PAULL, 1999). Ethanol concentrations but not acetaldehyde concentrations affected the formation of off-flavours. The current sensory note were not detected at the end of fruit storage in the gas mixture labelled as FAN.

Detection of traces of ethanol in ambient atmosphere

This study has shown that, in all cases, exposure to very low oxygen has the potential to change the aroma compounds in the ambient atmosphere. However, the amount of ethanol in the flesh of the fruit clearly increased, and a portion of this is released in gaseous form through the skin into the surrounding atmosphere (Fig. 6 and 7), and therefore this volatile was accumulated in the hermetically sealed chambers. Apart from the possibility of absorption by the chamber walls and the small fluctuations in consequence by flushing with nitrogen, it could be that the control of oxygen levels in the storage atmosphere depends on the fermentation rate (Fig. 8, 9, 10). Therefore it is desirable to know the content of ethanol as well as of the other measured compounds. The production of ethanol in anaerobic conditions (FAN atmosphere) increases in stored fruit from 101 μg $kg^{-1}h^{-1}$ to 550 μg $kg^{-1}h^{-1}$ over 16 to 27 days (Fig. 6), which practically constituted a straight-line increase. The ethanol released from fruit stored in ULO and CA atmospheres was accumulated in the ambient atmoshere at very low concentrations, two orders lower than in the FAN atmosphere (Fig. 7). The diffusion of gases into the ambient atmosphere depends on the actual rate of formation of anaerobic compounds in the fruit flesh and also the corresponding concentration in the surrounding atmosphere.

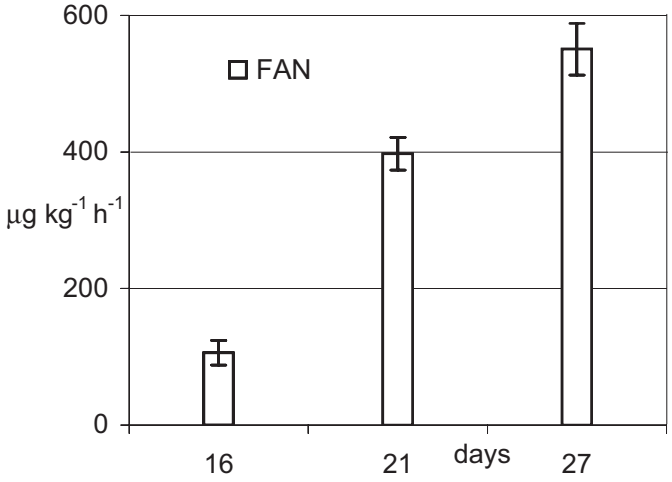

Fig. 6: Production of ethanol (EtOH) (μg $kg^{-1}h^{-1}$) released from intact fruit continuously stored in FAN – fluctuating anaerobiosis – 0.2 % O_2 + 0.9 % CO_2, after 16, 21 and 27 days (means and standard errors, n = 3).

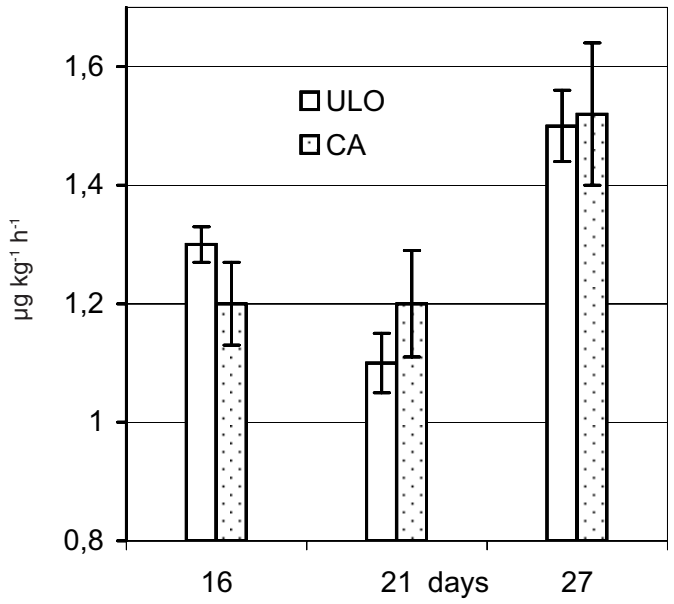

Fig. 7: Production of ethanol (EtOH) (µg $kg^{-1}h^{-1}$) released from intact fruit continuously stored in ULO – ultra-low oxygen – 1.0-1.2 % O_2 + 0.5 % CO_2 and CA – controlled atmosphere – 2.0-2.5 % O_2 + 6.5-7.0 % CO_2 after 16, 21 and 27 days (means and standard errors, n = 3).

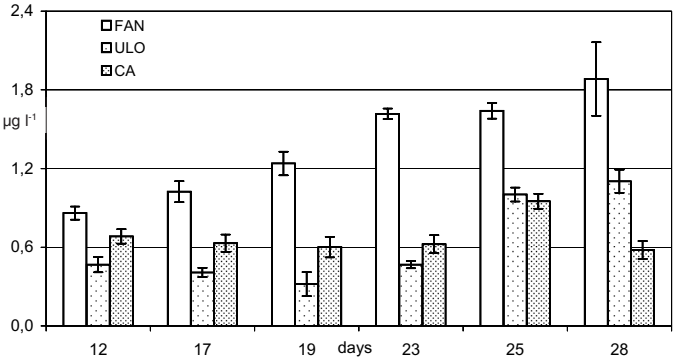

Fig. 8: Concentration of acetaldehyde (Act) (µg/l) in ambient atmosphere of storage room in FAN – fluctuating anaerobiosis – 0.2 % O_2 + 0.9 % CO_2, ULO – ultra-low oxygen – 1.0-1.2 % O_2 + 0.5 % CO_2, CA – controlled atmosphere – 2.0-2.5 % O_2 + 6.5-7.0 % CO_2 (means and standard errors, n = 3).

Traces of acetaldehyde and ethyl acetate in ambient atmosphere

Acetaldehyde, as a closely associated by-product of anaerobic glycolysis, was produced in the flesh at very much lower rates than ethanol, but the presence or absence of oxygen under the threshhold of aerobic metabolism had similar effects. The actual concentrations during the storage period essentially differentiated the treatments into two groups – very low oxygen atmosphere (FAN atmosphere) and the second group – ULO and CA atmospheres (Fig. 9). The same specificy the relationship applies to the production and subsequent release from intact fruit of ethyl acetate. This was detected at very small concentrations in the ambient atmosphere of the storage chamber, at traces from 20 to 80 nl l^{-1}, for all storage regimes (Fig. 10).

Acknowledgements

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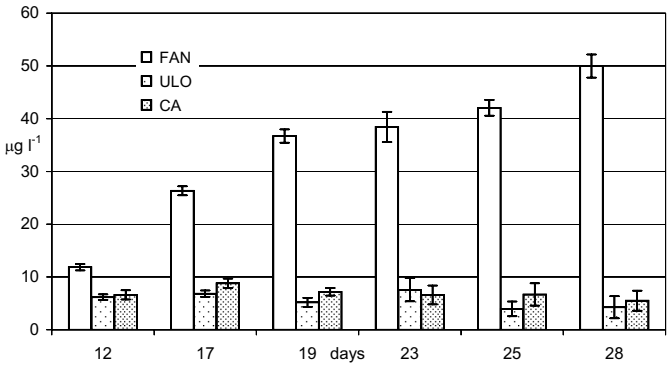

Fig. 9: Concentration of ethanol (EtOH) (µg/l) in ambient atmosphere of storage room in FAN – fluctuating anaerobiosis – 0.2 % O_2 + 0.9 % CO_2, ULO – ultra-low oxygen – 1.0-1.2 % O_2 + 0.5 % CO_2, CA – controlled atmosphere – 2.0-2.5 % O_2 + 6.5-7.0 % CO_2 (means and standard errors, n = 3).

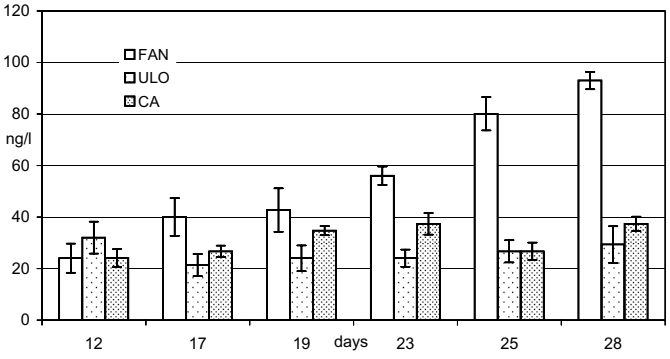

Fig. 10: Concentration of ethyl acetate (EtOAc) (ng/l) in ambient atmosphere of storage room in FAN – fluctuating anaerobiosis – 0.2 % O_2 + 0.9 % CO_2, ULO – ultra-low oxygen – 1.0-1.2 % O_2 + 0.5 % CO_2, CA – controlled atmosphere – 2.0-2.5 % O_2 + 6.5-7.0 % CO_2 (means and standard errors, n = 3).

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