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Postharvest Response of Apple Fruits after Storage in Extreme Gas Concentrations
II. The Occurence of Anaerobic Metabolites

Nachlagerverhalten von Apfelfrüchten nach dem Aufbewahren unter extremen Gaskonzentrationen II. Auftreten von anaeroben Metaboliten

J. Goliáš[1)] and H. Böttcher[2)]
([1)]Department of Postharvest Technology of Horticultural Products of the Mendel University of Agriculture and Forestry, Brno und [2)]Institute of Nutrition Sciences of the Martin-Luther-University, Halle-Wittenberg)

Summary

After a long-term storage the concentration of ethanol and acetaldehyde is characterized by changes dependent on the composition of the gas mixture. Regular atmosphere did not differ significantly from low-oxygen atmosphere with 1 % oxygen. This low concentration of oxygen, technically maintained, did not harm the fruits by inducing ethanol accumulation in the tissues.

The extreme atmosphere compositions (8 % CO_2 and 1 % O_2) resulted in an increased ethanol level in the tissues which were influenced by different degrees of ripening of fruits before their placement into cold-storage conditions. Less striking were the changes in acetaldehyde content. But higher values of acetaldehyde were observed in the extreme atmosphere with a high level of CO_2.

The effect of anaerobic conditions (0.2 % O_2) led to a striking production of ethanol which reached in the studied cultivars 630 or 900 mg l^{-1} in 20 days, or 25 days, respectively. Subsequent exposition of the fruits to air but still under cold-storage temperature resulted in an exponential decrease of ethanol which after 56 or 66 days dropped to half or even one third of its previous value. The speed of the oxidative degradation was much slower than in the cumulative phase.

Zusammenfassung

Nach Langzeitlagerung zeigten die Konzentrationen von Ethanol und Acetaldehyd Veränderungen in Abhängigkeit von der Zusammensetzung der Gasatmosphäre. Normal zusammengesetzte Atmosphäre wich nicht signifikant von der Atmosphäre mit geringem Sauerstoffgehalt von 1 % ab. Diese niedrige Konzentration von Sauerstoff schädigte die Früchte durch die induzierte Alkoholanreicherung im Gewebe nicht.

Extreme Gaszusammensetzung (8 % CO_2 und 1 % O_2) führte zu einem erhöhten Alkohollevel im Fruchtgewebe, welcher von den verschiedenen Reifezuständen der Früchte vor ihrer Einbringung in das Kaltlager beeinflußt war. Weniger auffällig waren die Veränderungen im Acetaldehyd-Gehalt. Aber höhere Werte von Acetaldehyd wurden bei der extremen Gaszusammensetzung mit einem hohen Gehalt an CO_2 beobachtet.

Die Wirkung anaerober Bedingungen (0,2 % O_2) führte zu einer zunehmenden Bildung von Ethanol, welche bei den untersuchten Sorten innerhalb von 20 bzw. 25 Tagen bis zu 630 oder 900 mg l^{-1} erreichte. Anschließende Lagerung der Früchte in Luft, aber noch unter Kaltlagertemperaturen, führte zu einer exponentiell verlaufenden Abnahme des Ethanol, welche nach 56 oder 66 Tagen auf die Hälfte oder sogar ein Drittel des ursprünglichen Wertes abfiel. Die Geschwindigkeit der oxidativen Degradation von Ethanol war bedeutend langsamer als die Anreicherung in der kumulativen Phase.

Introduction

Apple fruits are stored at cold storage temperature in controlled gas atmosphere in order to prolong their storagelife. In a typical controlled-atmosphere, the oxygen (O_2) content ranges within 2–3 % and carbon dioxide (CO_2) lower than 5 %. The aim of the last two decades was to achieve a further decrease of oxygen level <2 % in the form of ULO-storage. The decrease of the partial pressure of the oxygen in the ambient atmosphere slows down the ripening processes, which results in better pulp firmness, maintenance of the original skin colour, and lower acid consumption (STOW and GENGE 2000, GARCIA and STREIF 1993, BRACKMANN et al. 1994, RIZZOLO et al. 1997). Together with the lower oxygen, the increased concentration of carbon dioxide acts favourably towards slower metabolism as it is measured via respiration intensity (FIDLER and NORTH 1971, YERSLEY et al. 1997).

As low-oxygen atmosphere, one can regard any such gas mixture which makes it possible for the fruits to still effectively absorb oxygen in order to maintain the aerobic respiration.

The lowest oxygen limit (LOL) is the lowest critical value for storage gas atmosphere. The optimal storage atmosphere, at which the aerobic respiration is kept at a minimum which is still capable of precluding the anaerobic metabolism, occurs just above LOL (YERSLEY et al. 1996, GRAN and BEAUDRY 1993). If the aerobic respiration is activated, one of its products is ethanol, a not much volatile substance, which is only minimally released into the ambient atmosphere through the skin (CAMERON et al. 1995) and therefore cumulates in the pulp (PATTERSON and NICHOLS 1988).

Against the methodology of establishing the lowest oxygen concentration in the ambient atmosphere as

the concentration which does not stimulate the production of ethanol and acetaldehyde, there is the observation that traces of both metabolites are found even in sound and physiologically unharmed fruits (PEPPELENBOS and OOSTERHAVEN 1998).

The content of acetaldehyde has been connected with many post-harvest physiological disorders related to temperature, low-oxygen and high CO_2-level in ambient atmosphere (SMAGULA and BRAMLAGE 1977). Its physiological toxicity has been considered higher than that of cumulated ethanol (CHERVIN et al. 1996).

The present paper examines the effects of different levels of oxygen and CO_2 on the content of ethanol and acetaldehyde in the liquid portion of fruits after a long-term cold storage. Cultivars of apples stored in anaerobic conditions were observed during the rising concentration phase for both metabolites as well as the subsequent degradation phase when the fruits were exposed to normal air.

Materials and Methods

Fruits supply, treatment and storage

The experiments were carried out on 'Golden Delicious' and 'Idared' apples grown in the area round Halle/Saale. The fruits were harvested in 3 intervals separated by 7 days each. The first harvest date for both varieties was September 24 when the fruits were picked at pre-climacteric maturity according to the reduced of starch conversion on the cross section of the fruits. The second date was at optimal maturity. The fruits picked at the third harvesting date showed signs of ripening.

Within 18 hours after the harvest the fruits were put into gas-tight containers (210 litres) and the controlled atmosphere was established

Variant V1:	0 % CO_2 +	1 % O_2
Variant V8:	0.03 % CO_2 +	21 % O_2
Variant V10:	8 % CO_2 +	1 % O_2

The atmosphere was constantly monitored and the required gas concentration maintained. The necessary O_2 and N_2 supplied by an air separation device (ESO 3) which contains a molecular sieve as the active agent and by the addition of technical CO_2-gas. In order to measure the contents of the metabolites, the fruits from the third harvest date were used.

In another experiments the fruits of the cultivars 'Jonagold' and 'Golden Delicious' from the South Moravian region (commercial orchards of the co-operative in Nosislav) were harvested from 10-year-old trees on stock M9 at the optimal harvestdate according to the starch test.

All the fruits were picked on September 15. Immediately after cooling to 3 °C, the fruits were put into the gas mixture as follows for 20 days:

Variant AN:	0.1 % CO_2 +	0.2 % O_2
Variant ULO:	< 0.3 % CO_2 +	0.9 % O_2
Variant RA:	0.03 % CO_2 +	21 % O_2

After 20 days the fruits were exposed to a normal oxygen atmosphere at 3 °C.

The other two cultivars 'Melrose' and 'Idared' came from the same growing conditions and were harvested at the optimal maturity stage. They were put into the CA atmosphere on October 30 and kept for 25 days in the same concentrations of both gases as mentioned before. The gas mixtures were monitored hourly with the precision of 0.1 % of each gas (Dual Gas Analyser O_2/CO_2, model TS12E, Fa Arelco ARC, Fontenay, France).

Collection of samples, measurement of the substances in the liquid phase, statistic processing

At the beginning and the end of CA-treatment as well as in the intervals of introducing the regular atmosphere, a homogenous juice was separated from 6 fruits of each cultivar and frozen to –18 °C. The chromatographic analysis followed: The defrosted samples were filtered (25 mm Syringe Filters with Glass Pre-Filters, Polypropylene Housing, 0.2 mm Pore Size , Alltech,Associates, Inc., IL) and 1 ml of the non-diluted filtrate was injected into the packed column (length 1.2 m, the inner diameter 3 mm) filled with Porapak P (Waters Ass., Inc., Framingham, Mass.,USA). Into the injection space of the chromatograph, crushed teflon was added periodically to adsorb the ballast substances contained in the liquid sample.

GC-conditions: oven temperature 92 °C, detector temperature 150 °C, injection temperature 120 °C, carrier gas He (12 ml min^{-1}), FID, Varian 3400, Germany.

The quantitative study of acetaldehyde,ethanol and ethylacetate was carried out with absolute calibration. This methodology made it possible to get a graph showing the dependency between the amount of the standard substance and the size of the chromatographic peak. The calibration and the analyses were carried out under identical conditions. The results in graphs are expressed in mg l^{-1}.

Statistic was done at the basis of the evaluation of different storage conditions, harvest dates (by means of the standard error method) and quantification of the content during the gas treatment and subsequent storage in the normal atmosphere at 3 °C.

Differences of mean values were characterized by a significance-level of $p = 0.05$

Results

The influence of the harvest date

The measurement of acetaldehyde and ethanol began at the end of the main storage period after 7.5 months (Fig.1 to 3). For 'Golden Delicious', the content of acetaldehyde ranges within the interval between 0.11 and 0.35 mg l^{-1}, whereas 'Idared' is characterized by a significantly higher concentration (from 0.20 to 0.74 mg l^{-1}). The values for 'Jonagold' being comparable to those of 'Golden Delicious'. Under the chosen harvest dates, the patterns of acetaldehyde from the pre-climacteric period till the stage of mild ripening shows a slight decrease. This tendency concerns all three cultivars studied, the differences between the cultivar not being very striking. There is no indication of a dramatic increase of ripening on the tree or of physiological harm injury to the fruits during the storage.

According to the results of ethylene production of fruits of the same storage experiment which were already published (GOLIÁŠ et al. 2001) no deviating

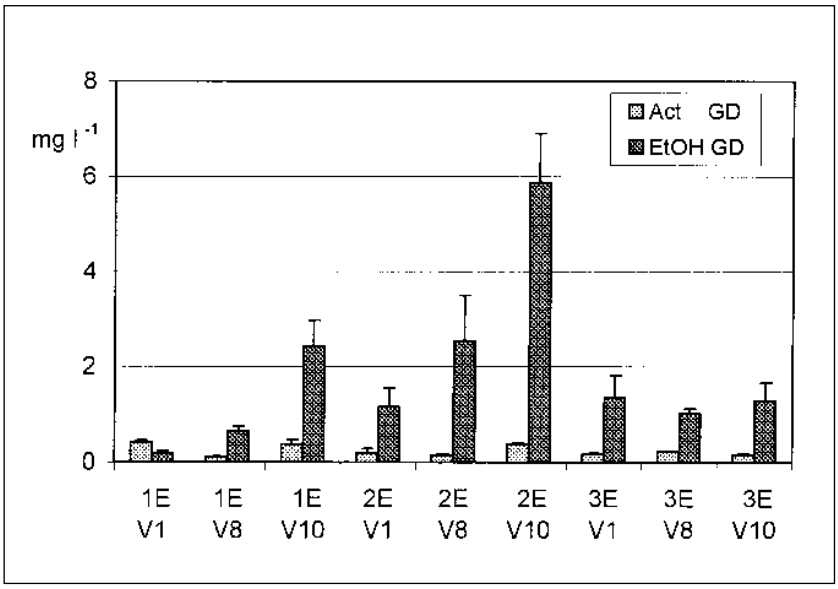


Fig. 1. The concentration of acetaldehyde (Act) and ethanol (EtOH) in 'Golden Delicious' (GD) apples after 7.5-month storage in different of gas mixtures [V1 (0 % CO_2 + 1 % O_2), V8 (0.03 % CO_2 + 21 % O_2) , V10 (8 % CO_2 + 1% O_2)] and for three different harvest dates [pre-climacteric fruits (1E), climacteric fruits (2E) and mild ripening fruits (3E]. Each value represents 6 fruits and vertical bars indicate SE, $P<0.05$.
Die Konzentration von Acetaldehyd (Act) und Ethanol (EtOH) in 'Golden Delicious' (GD) Äpfeln nach einer Lagerung von 7,5 Monaten in verschiedenen Gaszusammensetzungen [V1 (0 % CO_2 + 1 % O_2), V8 (0,03 % CO_2 + 21 % O_2), V10 (8 % CO_2 + 1 % O_2)] und für drei verschiedene Erntedaten [vorklimakterisch (1E), klimakterisch (2E) und reifebeginnend (3E)]. Jeder Wert steht für 6 Früchte, die senkrechten Linien kennenzeichnen die Standardabweichung SE, $p<0,05$.

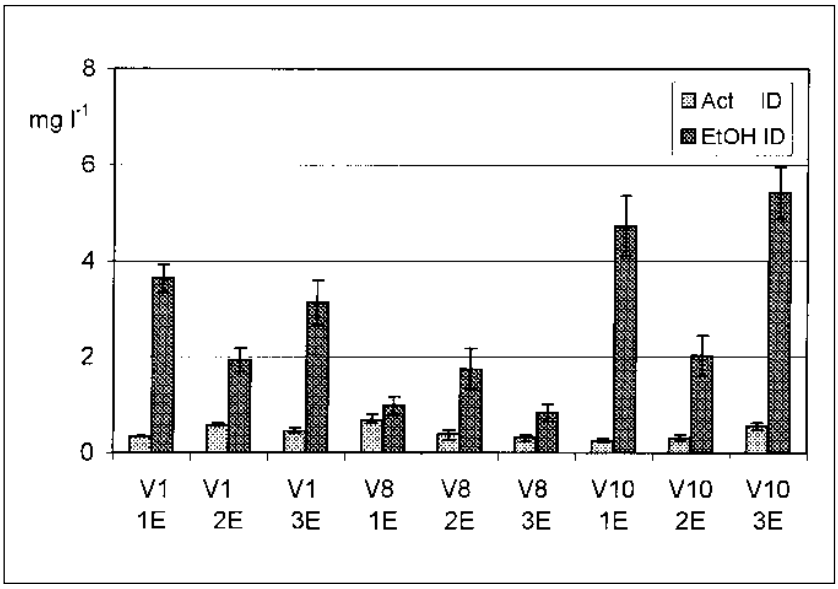


Fig. 2. The concentration of acetaldehyde (Act) and ethanol (EtOH) in 'Idared' (ID) apples after 7.5-month storage in different of gas mixture [V1 (0 % CO_2 + 1 % O_2), V8 (0.03 % CO_2 + 21 % O_2) , V10 (8 % CO_2 + 1% O_2)] and for three different harvest dates [pre-climacteric fruits (1E), climacteric fruits (2E) and mild ripening fruits (3E)]. Each value represents 6 fruits and vertical bars indicate SE, $P<0.05$.
Die Konzentration von Acetaldehyd (Act) und Ethanol (EtOH) in 'Idared' (ID) Äpfeln nach einer Lagerung von 7,5 Monaten in verschiedenen Gaszusammensetzungen [V1 (0 % CO_2 + 1 % O_2), V8 (0,03 % CO_2 + 21 % O_2), V10 (8 % CO_2 + 1 % O_2)] und für drei verschieden Erntedaten [vorklimakterisch (1E), klimakterisch (2E), und reifebeginnend (3E)]. Jeder Wert steht für 6 Früchte, die senkrechten Linien kennenzeichnen die Standardabweichung SE, $p<0,05$.

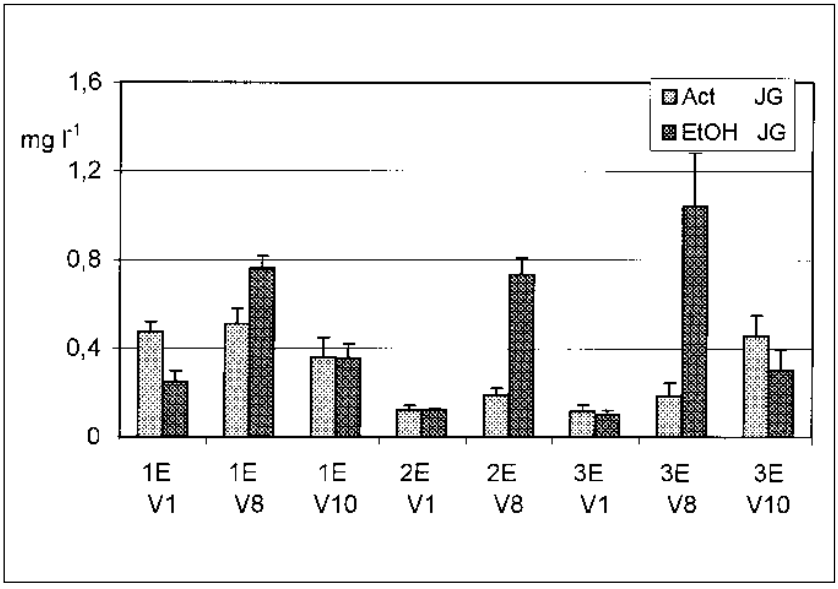


Fig. 3. The concentration of acetaldehyd (Act) and ethanol (EtOH) in 'Jonagold' (JG) apples after 7.5-month storage in different gas mixture V1 (0 % CO_2 + 1 % O_2), V8 (0.03 % CO_2 + 21 % O_2) , V10 (8 % CO_2 + 1% O_2)] and for three different harvest dates [pre-climacteric fruits (1E), climacteric fruits (2E) and mild ripening fruits (3E)]. Each value represents 6 fruits and vertical bars indicate SE, $P<0.05$.
Die Konzentration von Acetaldehyd (Act) und Ethanol (EtOH) in 'Jonagold' (JG) Äpfeln nach einer Lagerung von 7,5 Monaten in verschiedenen Gaszusammensetzungen [V1 (0 % CO_2 + 1 % O_2), V8 (0,03 % CO_2 + 21 % O_2), V10 (8 % CO_2 + 1 % O_2)] und für drei verschiedene Erntedaten [vorklimakterisch (1E), klimakterisch (2E), und reifebeginnend (3E)]. Jeder Wert steht für 6 Früchte, die senkrechten Linien kennenzeichnen die Standardabweichung SE, $p<0,05$.

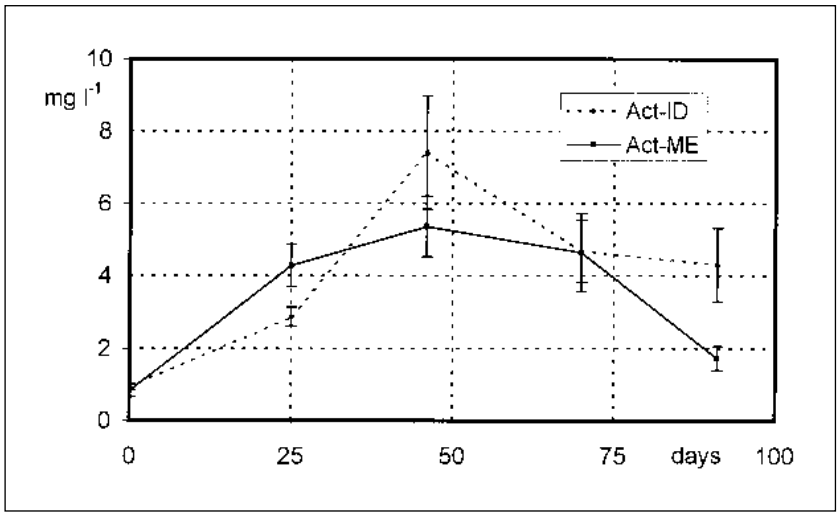


Fig. 4. The time pattern of acetaldehyde (Act) concentration in the ambient atmosphere stored under anaerobic conditions (0.2 % O_2) for 'Idared' (ID) and 'Melrose' (ME). This phase lasted for 25 days, subsequently the fruits were stored at cold-storage temperature and in the air. Each value represents 6 fruits and vertical bars indicate SE, $P<0.05$.
Der zeitliche Verlauf der Acetaldehyd-Konzentration in der Umgebungsluft bei anaeroben Bedingungen (0,2 % O_2) für 'Idared' (ID) und 'Melrose' (ME). Dieser Abschnitt dauerte 25 Tage, anschließend lagerten die Früchte bei Kaltlagertemperaturen und in normal zusammengesetzter Atmosphäre. Jeder Wert steht für 6 Früchte, die senkrechten Klammern kennzeichnen die Standardabweichung SE, $p<0,05$.

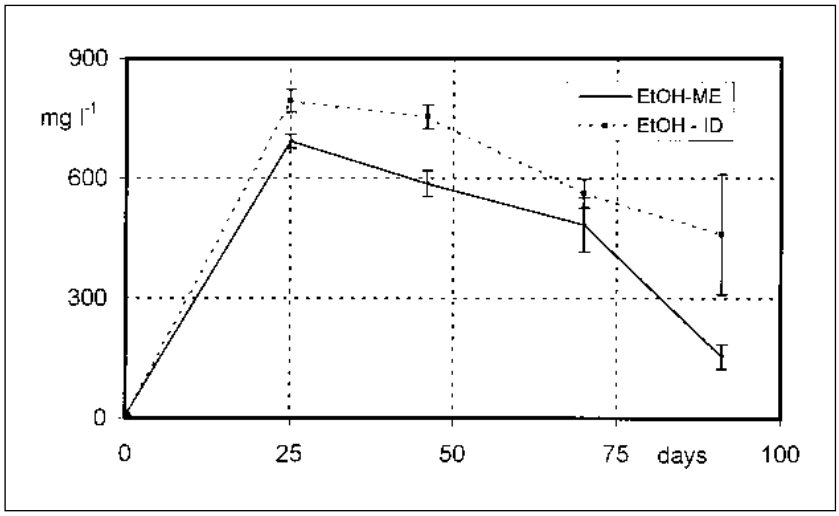


Fig. 5. The time pattern of ethanol concentration (EtOH) in the ambient atmosphere under anaerobic conditions (0.2 % O_2) for 'Idared' (ID) and 'Melrose' (ME). This phase lasted for 25 days, subsequently the fruits were stored at cold-storage temperature and in normal composed air. Each value represents 6 fruits and vertical bars indicate SE, $P<0.05$.
Der zeitliche Verlauf der Ethanol-Konzentration in der Umgebungsluft bei anaeroben Bedingungen (0,2 % O_2) für 'Idared' (ID) und 'Melrose' (ME). Dieser Abschnitt dauerte 25 Tage, anschließend lagerten die Früchte bei Kaltlagertemperaturen und in normal zusammengesetzter Atmosphäre. Jeder Wert steht für 6 Früchte, die senkrechten Klammern kennzeichnen die Standardabweichung SE, $p<0,05$.

senescence pattern were found in 'Golden Delicious'. In 'Idared' the production of the fruits stored in the air had a slower pattern of ethylene production.

Ethanol was detected both in preclimacteric and ripe fruits and, in different cultivars and storage atmosphere. It ranges from tenths of mg l^{-1} as far as 6–8 mg l^{-1} (Fig. 1 to 3). The effect of ripeness according to the harvest dates has been shown to have relatively higher values in the second harvest date.

The influence of the cultivar and storage conditions

The concentrations of acetaldehyde at the normal air atmosphere are identical for cultivar 'Golden Delicious' and 'Jonagold', while 'Idared' shows always higher values. In the high-CO_2 atmosphere (V10), there was no observation of extremes in contrast with high oxygen (V8) and in significantly low oxygen (V1) On the other hand, in low oxygen (V1), the acetaldehyde concentrations are comparably similar with regular atmosphere (V8).

The extreme atmosphere (V10), is differentiated only by the concentration of ethanol which is here always higher than in the variants in regular atmosphere (V8) and those with low oxygen (V1). 'Jonagold' shows permanently low ethanol levels in comparison to 'Golden Delicious' and 'Idared': these levels range up to 1 mg l^{-1} (Fig. 3).

The production of acetaldehyde and ethanol in freshly harvested apple fruits under anaerobic postharvest conditions (The time pattern of the production) and during the phase of air storage.

The high ethanol production in the tissues which are kept under anaerobic conditions is a result of the anaerobic glycolytic process. The concentration of oxygen in anaerobic conditions was 0.2 % O_2 and the exposition for the treatment of 'Idared' and 'Melrose' was 25 days (Fig. 4 and 5) and 20 days for 'Golden Delicious' and 'Idared' (Fig. 6 and 7). During subsequent time the fruit were kept in air.

The production of acetaldehyde did not coincide with the date at which the anaerobic conditions were discontinued but rose still at the time of them being exposed to air.

The differences in the content of acetaldehyde are statistically significant (means ±SE, $P<0.05$). For 'Idared' and 'Melrose' the concentration of acetaldehyde did not surpass 8 mg l^{-1} (Fig.4). The accumulation of ethanol began with the trace concentration 2–3 mg l^{-1} before the gas treatment in 0.2 % O_2. The response of the cultivars is similar: the speed of accumulation was practically identical but it differs in the nominal value. 'Idared' and 'Melrose' showed some significant differences, on the other hand, 'Golden Delicious'

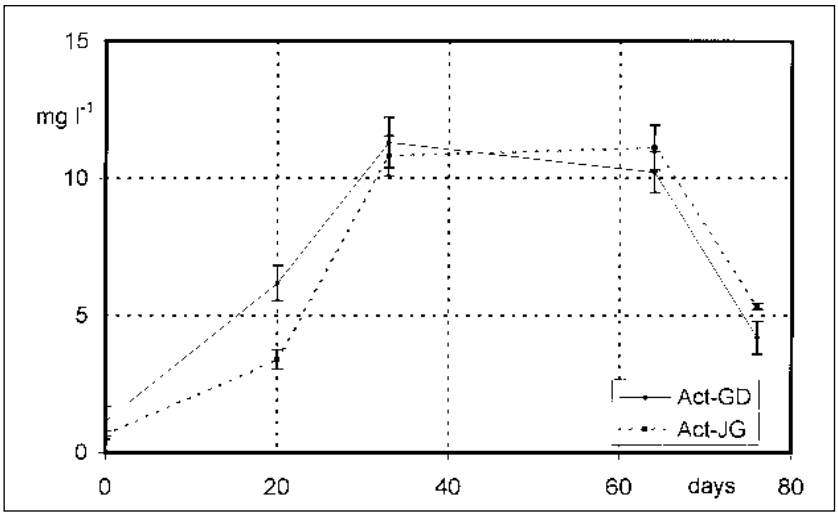


Fig. 6. The time pattern of acetaldehyde (Act) concentration in the ambient atmosphere under anaerobic conditions (0.2 % O_2) for 'Golden Delicious' (GD) and 'Jonagold' (JG). This phase lasted for 20 days, subsequently the fruits were stored at cold-storage temperature and in normal composed air. Each value represents 6 fruits and vertical bars indicate SE, $P<0.05$.
Der zeitliche Verlauf der Acetaldehyd-Konzentration in der Umgebungsluft bei anaeroben Bedingungen (0,2 % O_2) für 'Golden Delicious' (GD) und 'Jonagold' (JG). Dieser Abschnitt dauerte 25 Tage, anschließend lagerten die Früchte bei Kaltlagertemperaturen und in normal zusammengesetzter Atmosphäre. Jeder Wert steht für 6 Früchte, die senkrechten Klammern kennzeichnen die Standardabweichung SE, $p<0,05$.

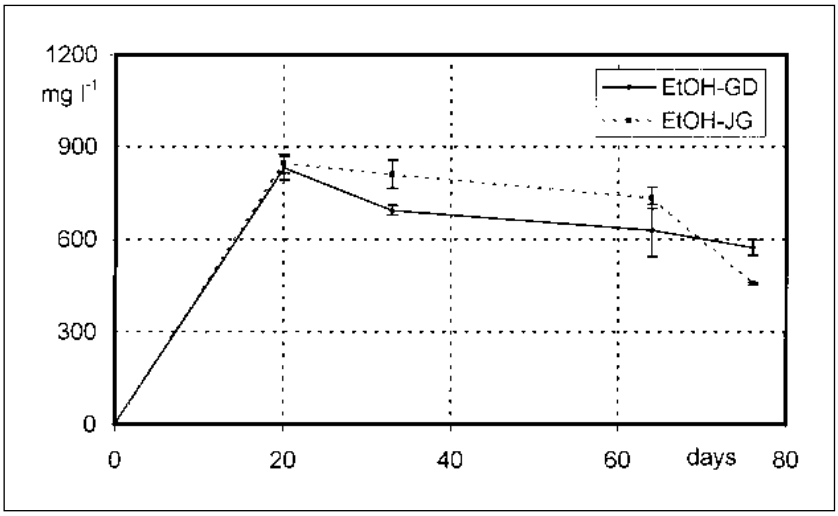


Fig. 7. The time pattern of ethanol concentration (EtOH) in the ambient atmosphere under anaerobic conditions (0.2 % O_2) for 'Golden Delicious' (GD) and 'Jonagold' (JG). This phase lasted for 20 days, subsequently the fruits were stored at cold-storage temperature and in normal composed air. Each value represents 6 fruits and vertical bars indicate SE, $P<0.05$.
Der zeitliche Verlauf des Ethanolgehaltes in der Umgebungsluft bei anaeroben Bedingungen (0,2 % O_2) für 'Golden Delicious' (GD) und 'Jonagold' (JG). Dieser Abschnitt dauerte 25 Tage, anschließend lagerten die Früchte bei Kaltlagertemperaturen und in normal zusammengesetzter Atmosphäre. Jeder Wert steht für 6 Früchte, die senkrechten Klammern kennzeichnen die Standardabweichung SE, $p<0,05$.

and 'Jonagold' have identical values at the end of the cumulative phase

The degradation of acetaldehyde and ethanol in the tissues during their placement in normal air.

When fruits were transferred to air, the concentration of ethanol in the tissue decreased exponentially. This degradation phase lasted at the cold-storage temperature for 56 days (Fig. 4 and 5), and 66 days (Fig. 5 and 6). The ethanol level, however, remainded higher than in ventilated fruits. The degradation of ethanol depends on the cultivar. Only during the air ventilation phase (Fig. 4 to 6) the drop of acetaldehyde concentration was statistical significant.

Discussion

Next to the temperature as a factor influencing the metabolic activity, the composition of the gas mixture is a significant additional factor for the decrease of the metabolic activity of the fruits stored after the harvest. This is accentuated by the right choice of the picking date (BOHLING and HANSEN 1985, CHEN et al. 1989).

The knowledge of the low oxygen limit is a critical presupposition for the optimal control of the gas composition of the storage atmosphere. The atmosphere which occurs just above this limit is regarded as the optimal one. In such an atmosphere the aerobic oxidation is at its lowest and, at the same time, no anaerobic products appear (YEARSLEY et al. 1996, PEPPELENBOS and OOSTERHAVEN 1998). The traditional methods involved measurements of the speed of CO_2 production on differential levels of the partial oxygen pressure (pO_2). FIDLER and NORTH (1967) demonstrated that the respiration quotient (RQ) for apple tissue is not affected when, simultaneously, the oxygen pressure in the ambient atmosphere goes from 21 kPa to 2 kPa. If it is decrease still further, then a progressive transition takes place because the partial carbon dioxide will grow. As further indicators the concentration of acetaldehyde, ethanol and ethyl acetate in the gas space above the fruit, into which they get through the diffusion across the intact skin, can be used (GRAN and BEAUDRY 1993, CAMERON et al. 1995, SMYTH et al. 1999).

The concentration of acetaldehyde and ethanol are the results of a long-term storage of apple fruit in different gas mixtures. One of these contains only about 1 % O_2 (V 1), a very low and physiologically critical level. This controlled atmosphere could potentially induce an increased production of both substances. But we were able to show that the concentration of ac-

etaldehyde for 'Golden Delicious', 'Idared' and 'Jonagold', on contrary, are fully comparable to storage in air (V 8). Ethanol concentration in the atmosphere was only doubled for apple variety 'Idared'.

However, when the concentration of CO_2 in the atmosphere was increased to 8 % (V 10), the level of two metabolites studied were in most cases significantly higher, especially for 'Golden Delicious' and 'Idared', but not for 'Jonagold'. Nevertheless, the rise of the values was not so extremely high. In fact, they did not surpress 8 mg l^{-1} . On the contrary, the highest values for acetaldehyde were determined at 0.5 mg l^{-1}.

After introduction of typical anaerobic conditions with oxygen level at 0.2 % in the surrounding atmosphere in other trials, we were able to bring about a high production of acetaldehyde, an ethanol a much shorter period of 20 or 25 days. The concentrations reached were between 600 to 900 mg l^{-1} related to the the cultivar studied. According to PATTERSON and NICHOLS (1988) it is possible to expect a linear growth of the concentration to values of 5 800 µl l^{-1} ethanol in the apple tissues after 14 weeks, given the presence of null oxygen in the ambient atmosphere. On the other hand, only a mild increase of the oxygen concentration to 0.5 % and 1 % O_2 has caused a higher concentration of this component, but only to 354 µl l^{-1}.

Our results show tentatively that it is the rest of ethanol in the tissue subsequently degraded by oxidative metabolism which leads to the first signs of physiological injury (oxygen stress). In 'Golden Delicious' brown spots of the size of 1–2 cm^2 were observed on the surface, each fruit having one or two such spots. Various technological ways of bringing about the oxygen stress are thus connected to the physiological injury of apple fruits (LITTLE and PEGGIE 1987, LIDSTER et al. 1987).

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Anschrift der Verfasser: Prof. Dipl.-Ing. J. Goliáš, DrSc, Institut für Nacherntetechnologie von gärtnerischen Produkten der Mendel Universität für Landwirtschaft und Forstwirschaft, Valticka-Str. 337, CZ-69144 Lednice, Tschechische Republik, e-mail: golias@mendelu.cz und Prof. Dr. H. Böttcher, Institut für Ernährungswissenschften der Landwirtschaftlichen Fakultät, Emil Abderhalden-Str. 26, D-06108 Halle (Saale).