Tolerance of plum (*Prunus domestica* L.) fruits stored in low-oxygen atmosphere

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ABSTRACT: Plum fruits of the cultivars Stanley and Valjevka picked at the beginning of climacteric were stored in different storage atmospheres for 31 days at 3°C. The relations between the O_2 and CO_2 content during this period and after removal from the gas mixture to ethanol, acetaldehyde, non-volatile compounds and some textural values of fruits were investigated. Concentrations of ethanol in the flesh were related to levels of oxygen and CO_2 in ambient atmosphere. In anaerobic conditions (< 0.2% O_2) ethanol reached 1,109 mg/l for the cultivar Valjevka and 628 mg/l for Stanley. The results of single fruit analysis showed a steeply increasing concave curve of ethanol production in the period of anaerobic conditions, followed by the phase of a drop of the production rate in air stored fruit. The concentration of oxygen at a level of 0.9% (ultra low oxygen – ULO) does not physiologically harm the tissues of plums by producing mostly negligible content of ethanol and acetaldehyde, but an ethanol increase to half concentration after 31 days was observed to compare with anaerobic conditions in the cultivar Valjevka. From this aspect plums seem to be relatively sensitive to low oxygen. The post-storage period was extended up to 53 to 63 days, respectively. The senescence caused an increase in ethanol production rate that was exponentially increased after 20 days of cold storage atmosphere. The final concentration after 53 days was still higher for cv. Valjevka than for cv. Stanley at the respective content of 828 mg/l and 498 mg/l. Skin firmness was differentiated for both cultivars, and softness was higher for the cultivar Valjevka.

Keywords: ethanol; acetaldehyde; low oxygen; plums (*Prunus domestica* L.); firmness

Low oxygen storage of plums (*Prunus domestica* L.) could be an important commercial practice that slows down respiration rate and maintains fruit quality for a longer period than air storage. Concerning plums that are not stored extensively and for long storage periods cultivars with better firm-fleshed fruits are more convenient.

Low-temperature storage is beneficial for slowing down the ripening process in plums. External influences, such as temperature, are able to change the quality of the fruit significantly (PEIRS et al. 2000). Well-matured plum cultivars can usually be stored satisfactorily for 1 month at –1°C to 0°C with 90 to 95% relative humidity. Storage beyond 1 month often results in flesh browning and abnormal flavours (ASRAE 1968). SEKSE (1988, 1989) reported major physiological responses by carbon dioxide and ethylene evolution from plums during the last part of the growing season with a distinguished typical climacteric respiration pattern. Ethylene production started at the time minimum of carbon dioxide and increased sharply, approximately overlapping the carbon dioxide peak.

Post-harvest storage of plums is limited by the development of physiological disorders (BEN, GAVENDA 1992). Internal flesh browning appears due to enzymatic oxidation of polyphenols and gel breakdown in flesh caused by the presence of large water-soluble pectins that bind water into gel (TAYLOR et al. 1993; LURIE et al. 1997). Optimal storage atmosphere is based on physiological tolerance to O_2 and CO_2 (according to BEAUDRY 1993, 1999) as undesirable responses to the induction of fermentation.

If oxygen falls below the supporting aerobic respiration, glycolytic conversion of pyruvate to acetaldehyde and ethanol occurs (CHERVIN et al. 1996; KO et al. 1996). Tissue tolerance to anaerobic conditions may be variable, extended exposure to these conditions leads to tissue fermentation, browning, off-flavour and therefore a loss of economic value.

The objective of this study was to investigate fruit ripening of plum cultivars as related to the lower oxygen and minimal elevated carbon dioxide concentration during cooling storage.

MATERIALS AND METHODS

Source of fruit and storage procedures

Freshly harvested plums were obtained from a commercial orchard in Stošíkovice, South Moravian region. Fruit of cv. Stanley and Valjevka were picked at blue stage development (a few days before common commercial picking time). Maturity stage definitions are linked to other characteristics such as soluble solids, titratable acidity and tissue rheological value; they are

Supported by Ministry of Agriculture of the Czech Republic (Grant No. 1048).

Table 1. Anaerobic metabolites, soluble solids and titratable acidity at the beginning of storage and after 31 days in different oxygen and carbon dioxide content regimes followed by 53 days and 68 days in air in cv. Stanley*

Act (mg/l)			EtOH (mg/l)			SS (°Rf)			TA (g/l)		
treatment	time	$x \pm$ SE	treatment	time	$x \pm$ SE	treatment	time	$x \pm$ SE	treatment	time	$x \pm$ SE
IN	0	4 ± 1ab	IN	0	20 ± 4a	IN	0	14 ± 0.4abc	IN	0	0.73 ± 0.03ab
ULO	31	18 ± 5b	ULO	31	292 ± 69bc	ULO	31	13 ± 0.5ab	ULO	31	0.71 ± 0.02ab
AN	31	4 ± 1a	AN	31	629 ± 36d	AN	31	12 ± 0.3a	AN	31	0.82 ± 0.03b
RA	31	15 ± 1ab	RA	31	61 ± 4a	RA	31	15 ± 01bc	RA	31	0.73 ± 0.4ab
ULO	53	24 ± 4ab	ULO	53	120 ± 53ab	ULO	53	13 ± 0.1ab	ULO	53	0.71 ± 0.04ab
AN	68	25 ± 4ab	AN	68	232 ± 19ab	AN	68	16 ± 0.4c	AN	68	0.71 ± 0.01ab
RA	53	111 ± 61c	RA	53	498 ± 67bc	RA	53	14 ± 0.6abc	RA	53	0.60 ± 0.03a
Treatment		ns			**			**			*
Time		*			**			**			**
Treatment × time		ns			*			**			ns

Two-way ANOVA: ** $P < 0.01$, * $P < 0.05$, ns = not significant
*Values are means and standard errors calculated from six plum fruits subjected to treatment
Variants with no statistical difference are indicated by common letters (a, b, c, d)

Table 2. Textural parameters at the beginning of storage and after 31 days in different oxygen and carbon dioxide content regimes followed by 53 days and 68 days in air in cv. Stanley

Skin (MPa)			Flesh (MPa)			Firmness (MPa.s)		
treatment	time	$x \pm$ SE	treatment	time	$x \pm$ SE	treatment	time	$x \pm$ SE
IN	0	0.81 ± 0.03b	IN	0	0.08 ± 0.01bcd	IN	0	16.27 ± 0.47b
ULO	31	0.78 ± 0.05b	ULO	31	0.09 ± 0.00cd	ULO	31	15.24 ± 1.18b
AN	31	0.88 ± 0.03b	AN	31	0.11 ± 0.01d	AN	31	17.62 ± 1.00b
RA	31	0.35 ± 0.05a	RA	31	0.03 ± 0.00a	RA	31	6.75 ± 1.16a
ULO	53	0.47 ± 0.03a	ULO	53	0.05 ± 0ab	ULO	53	9.38 ± 0.56a
AN	68	0.73 ± 0.01b	AN	68	0.05 ± 0.01ab	AN	68	17.12 ± 0.89b
RA	53	0.41 ± 0.06a	RA	53	0.06 ± 0.00abc	RA	53	9.97 ± 1.60a
Treatment		**			**			**
Time		**			**			**
Treatment × time		*			**			*

Two-way ANOVA: ** $P < 0.01$, * $P < 0.05$, ns = not significant
*Values are means and standard errors calculated from six plum fruits subjected to treatment
Variants with no statistical difference are indicated by common letters (a, b, c, d)

Table 3. Anaerobic metabolites, soluble solids and titratable acidity at the beginning of storage and after 31 days in different oxygen and carbon dioxide content regimes followed by 53 days and 68 days in air in cv. Valjevka

Act (mg/l)			EtOH (mg/l)			SS (°Rf)			TA (g/l)		
treatment	time	$x \pm$ SE	treatment	time	$x \pm$ SE	treatment	time	$x \pm$ SE	treatment	time	$x \pm$ SE
IN	0	1 ± 0.3a	IN	0	28 ± 4a	IN	0	18 ± 0.1ab	IN	0	0.94 ± 0.4c
ULO	31	7 ± 1.2a	ULO	31	915 ± 23de	ULO	31	18 ± 0.3ab	ULO	31	0.89 ± 0.03bc
AN	31	5 ± 1.1a	AN	31	1,109 ± 34e	AN	31	17 ± 1.1a	AN	31	0.91 ± 0.02bc
RA	31	21 ± 2.2a	RA	31	117 ± 39a	RA	31	20 ± 0.8ab	RA	31	0.90 ± 0.06bc
ULO	53	47 ± 14.1a	ULO	53	475 ± 78b	ULO	53	19 ± 0.5ab	ULO	53	0.70 ± .0.05ab
AN	68	46 ± 10.6a	AN	68	699 ± 44c	AN	68	18 ± 1.0ab	AN	68	0.66 ± 0.05a
RA	53	55 ± 25.8a	RA	53	826 ± 16cd	RA	53	21 ± 0.9b	RA	53	0.70 ± 0.07ab
Treatment		ns			**			*			ns
Time		**			**			ns			**
Treatment × time		ns			ns			ns			ns

Two-way ANOVA: ** $P < 0.01$, * $P < 0.05$, ns = not significant
*Values are means and standard errors calculated from six plum fruits subjected to treatment
Variants with no statistical difference are indicated by common letters (a, b, c, d)

Table 4. Textural parameters at the beginning of storage and after 31 days in different oxygen and carbon dioxide content regimes followed by 53 days and 68 days in air in cv. Valjevka

Skin (MPa)			Flesh (MPa)			Toughness (MPa.s)		
treatment	time	$x \pm$ SE	treatment	time	$x \pm$ SE	treatment	time	$x \pm$ SE
IN	0	1.00 ± 0.02c	IN	0	0.09 ± 0.00b	IN	0	21.8 ± 0.58c
ULO	31	0.77 ± 0.06b	ULO	31	0.09 ± 0.01b	ULO	31	16.2 ± 1.38bc
AN	31	0.78 ± 0.05b	AN	31	0.08 ± 0.02b	AN	31	16.0 ± 1.95b
RA	31	0.37 ± 0.03a	RA	31	0.04 ± 0.00a	RA	31	8.9 ± 0.75a
ULO	53	0.65 ± 0.05b	ULO	53	0.05 ± 0.01ab	ULO	53	15.0 ± 51.62b
AN	68	0.78 ± 0.03b	AN	68	0.07 ± 0.00ab	AN	68	19.8 ± 0.46bc
RA	53	0.27 ± 0.03a	RA	53	0.02 ± 0.00a	RA	53	7.2 ± 0.62a
Treatment		**			**			**
Time		ns			*			ns
Treatment × time		ns			ns			ns

Two-way ANOVA: ** $P < 0.01$, * $P < 0.05$, ns = not significant
*Values are means and standard errors calculated from six plum fruits subjected to treatment
Variants with no statistical difference are indicated by common letters (a, b, c, d)

summarised in Tables 1 to 4. Fruits were cooled to 3°C within half a day of receipt and placed in 400 litre gas tight containers maintained in the following gas conditions: anaerobic AN (0.2% oxygen and 0.2% carbon dioxide), ultra low oxygen ULO (0.9% oxygen and 0.2% carbon dioxide), regular atmosphere RA (21% oxygen and 0.03% carbon dioxide). To achieve the desired concentration of O_2, flushing with N_2 was carried out. Carbon dioxide was removed using a caustic soda solution, except when ULO was required, nitrogen was then allowed to flush out excess carbon dioxide.

The concentrations of CO_2 and O_2 were continuously monitored by gas analysis (Arelco, ARC, France) and connected to a process computer. Gas concentration was checked each hour. Sub-samples of plums were removed at intervals of 10 days for the measurement of ethanol and acetaldehyde. At the main time of storage there was no need in the container to re-establish atmosphere conditions.

After 31 days in storage, each container was opened and 20 kg-lot (subsequently divided into four replications) from each container was transferred to the air atmosphere. The fruits were stored for additional 53 and 68 days, respectively, then removed and held at 20°C for up 1 hour before measuring. After 31, 53 and 68 days of the cold storage phase fruits were held for 3 days at 16°C to estimate internal browning.

Measurement of substances in liquid phase, statistical processing

At the beginning and at the end of all treatments as well as in the intervals of introducing atmosphere, homogeneous juice was separated from 6 fruits of two cultivars and frozen to –18°C. Chromatographic analysis followed: 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 µl of the undiluted filtrate was injected into a packed column (length 1.2 m, the inner diameter 3 mm) filled with Porapak P (Watters Ass., Inc., Framingham, Mass., USA). In the injection space of the chromatograph, crushed teflon was added periodically to adsorb the ballast substances contained in the liquid sample.

GC conditions were as follows: oven temperature 92°C, detector temperature 110°C, injection temperature 120°C and analysed with a gas chromatograph with FID (Chrom 5, Laboratory Equipment, Prague). Gas flow rates were 50 ml/min for H_2, 35 ml/min for He as carrier gas and 300 ml/min for air, respectively. The quantitative study of acetaldehyde and ethanol was carried out with absolute calibration and expressed in mg/l for each compound.

Fruit firmness was determined on 6 fruit samples on a multipurpose penetrometer (Multipurpose equipment for mechanical properties, TU Brno, Czech Republic) to test the opposite, sides of fruits. Titratable acidity (TA) and soluble solids (SS) were measured in the flesh of the fruit as juice extracted from the flesh of each fruit. In Figs. 1 to 5 vertical bars indicate standard error and each point is the mean of 6 replications.

Data from all treatments and cultivars were summarised by an analysis of variance, and the means and SE are presented in Tables 1–4. Differences in mean values were characterised by a significance level of $P = 0.05$ and the means were compared by Tukey's test to separate treatment means.

RESULTS AND DISCUSSION

Changes in ethanol accumulation in gas mix storage and subsequent phase in air

There are physiological relevances with the adequate storage procedures between increased fermentation metabolites and tissue stability to ethanol stress. Etha-

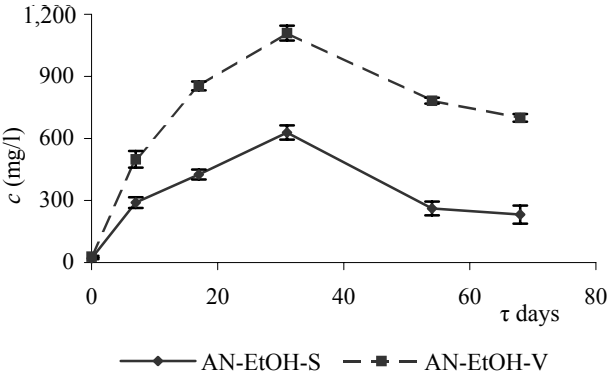

Fig. 1. The time pattern of ethanol (EtOH) concentration in the flesh of cv. Valjevka and Stanley plums exposed to anaerobic environment (0.2% O_2 and 0.2% CO_2)

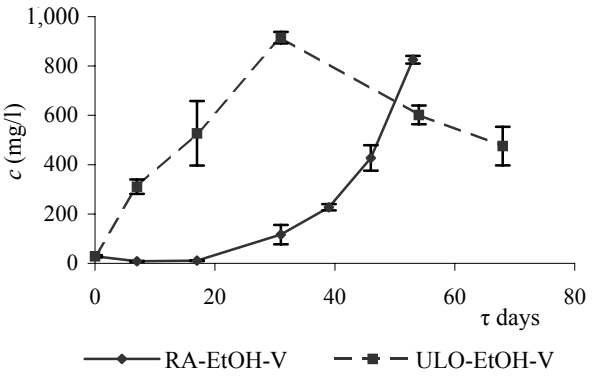

Fig. 3. The time pattern of ethanol (EtOH) concentration in the flesh of cv. Valjevka plums exposed to ULO (ultra low oxygen 0.9% O_2 and 0.2% CO_2) and RA (regular atmosphere 21% O_2 and 0.03% CO_2) at 3°C for 68 and 53 days, respectively

nol data shows active fermentation in the fruit tissue at O_2 levels at or below the extinction point, indicating anaerobic conditions (Fig. 1). The concentration of oxygen anaerobic conditions was 0.2% O_2 and exposition for the treatment of both cultivars was 31 days. The high ethanol production in the tissue is a result of the anaerobic glycolytic process. The cultivar differentiated only by the concentration of ethanol, which was higher for the cultivar Valjevka than in variants in ultra low atmosphere and regular atmosphere (Fig. 2). Stanley showed permanently lower ethanol levels in comparison with Valjevka: the levels ranged up to 1,100 mg/l (Fig. 1). For a subsequent period the fruits were kept in air. Exposition of fruits to air, but still under cold- storage temperature, resulted in an exponential decrease in ethanol which dropped to a half or a third of its previous value. Lower rates of ethanol production were also observed in ULO treatment, but the values in 31 days were a half of anaerobic conditions. Those in air-stored plums increased steadily until 53 days of storage (Fig. 4). The speed of oxidative degradation was much slower than in the cumulative phase. However, this does not directly reflect the tissue damage and the loss of value that may be expected when fruits are stored below the lower oxygen limit. Therefore the occurrence of fermentation itself cannot be a direct criterion for selecting an optimal storage concentration in plums. STREIF (1987) established the best storability of Bühler Frühzwetschen at 1% O_2 and 12% CO_2.

Senescence of fruits stored in air

Variations in ethanol production also occurred through the onset of storage periods, and these changes were largely correlated with cultivar properties (Table 1 and 3). In contrast, ethanol biosynthetic activity of air-stored plums remained relatively high during the storage after 20 days (Fig. 4).

The accumulation of ethanol began with the trace concentration of 20 mg/l (for cv. Stanley) and 28 mg/l (for cv. Valjevka) before gas treatment. The response of cultivars was similar: the trend of accumulation was practically identical, but the speed was higher for cv. Valjevka. Ethanol accumulation had an exponential pattern and nearly 20 days concentration was equal for both cultivars (Figs. 2–4). According to the results of ethanol production of fruits in the same storage experi-

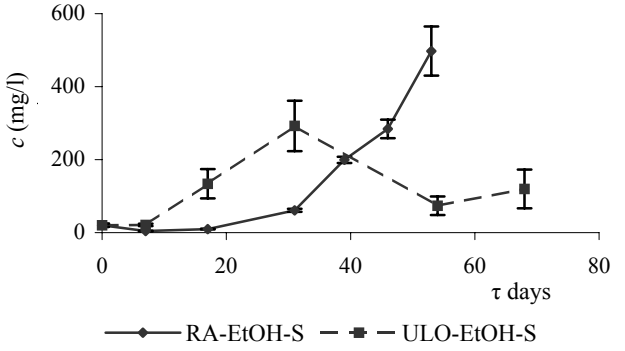

Fig. 2. Effect of O_2 level on ethanol content in the flesh of cv. Stanley exposed to ULO (ultra low-oxygen 0.9% O_2 and 0.2% CO_2) and RA (regular atmosphere 21% O_2 and 0.03% CO_2) at 3°C for 68 and 53 days, respectively

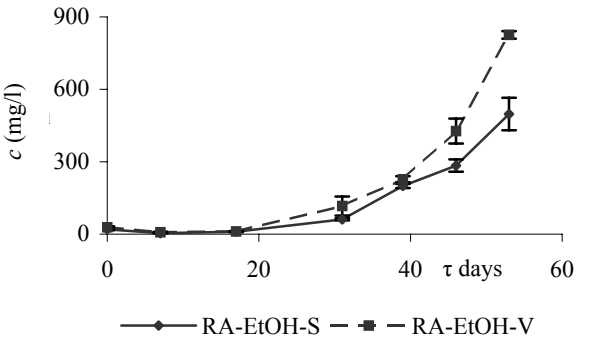

Fig. 4. The time pattern of ethanol (EtOH) concentration in the flesh of cv. Valjevka and Stanley plums exposed to RA (regular atmosphere 21% O_2 and 0.03% CO_2) at 3°C for 53 days

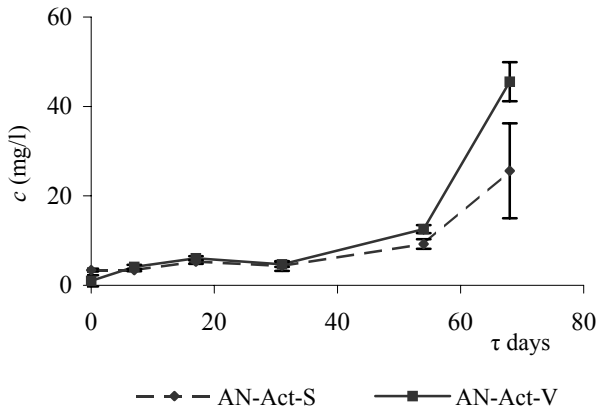

Fig. 5. The time pattern of acetaldehyde (Act) concentration in the flesh of cv. Valjevka and Stanley plums exposed to anaerobic environment (0.2% O_2 and 0.2% CO_2)

ment (fruits were stored in air) senescence showed as an elevated value after 20 days in cold storage.

Too much confidence should not be placed on the appearance and condition of the fruit while it is in storage, as more deterioration, decay, shrivelling and internal browning may take place within 3 days after removal from storage than during the whole storage period. After exposure during this time at 16°C, no changes developed regarding internal browning, but partial shrivelling occurred.

Acetaldehyde evaluation in storage treatment

Low oxygen concentrations are known to produce such accumulation, hence increases in acetaldehyde and ethanol are often taken as indication of fermentation in the tissue (SMAGULA, BRAMLAGE 1977). Acetaldehyde content showed a higher increase during all phases of gas mixture conditions. The fruits that were stored in air appeared to have a steady increase in acetaldehyde content, resulting in relatively high levels after 31 days to 53 or 68 days of storage, respectively (Fig. 5), despite an increase in ethanol content during the last phase of storage. It should be mentioned that the conversion of acetaldehyde to ethanol is suppressed under lower oxygen concentration within 53 days of storage (Fig. 5). A similar trend was observed in the delaying of AN and ULO plums, which showed an increase in acetaldehyde content up to 47 mg/l until 68 days of storage. Stepwise accumulation of acetaldehyde may be considered a part of the ripening syndrome, but the differences between all treatments were not significant, except the storage time (Tables 1 and 3).

Content of titratable acidity and soluble solids

Soluble solids – SS – were obtained in flesh mass. During the whole experiment, the reserve compounds were not measured as soluble solids significantly changed, but there were differences between both cultivars (Tables 1 and 3). Storage conditions had a statistically significant influence on soluble solids in the cultivar Stanley only (Table 1). Titratable acidity measured in flesh mass was significantly influenced by the time of storage in air, and high metabolic consumption was confirmed by RA variant for the cultivar Stanley.

Fruit softening

There is a number of parameters that consumers associate with ripening, such as firmness, flavour, texture and colour. Firmness is evidently affected by the onset of obvious ripening. The retarded firmness loss corresponded with inhibited oxygen concentration in ambient atmosphere. Fruits from all treatments ripened normally with almost no physiological storage disorders, except for a small amount of flesh browning during the end of shelf life in air. Softening of fruit tissue is associated with changes in the degree of polymerisation of pectic substances resulting in decreased tissue cohesion. Long-term temperature storage at low oxygen concentration led to high maintenance of textural value assessments as firmness of skin and flesh (Tables 2 and 4). Firmness of all treatments in modified gas mixture was significantly higher than in fruits in RA conditions coincident to air composition. The influence of time and treatment on cultivar properties was significantly higher in cv. Stanley than cv. Valjevka (Tables 2 and 4). Therefore, further study is needed to determine the sequence of oxygen/carbon dioxide concentration leading to plum softening.

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Received for publication September 4, 2003
Accepted after corrections January 5, 2004

Tolerance plodů švestek k nízkému obsahu kyslíku při skladování

ABSTRAKT: Švestky dvou odrůd (Stanleyova a Valjevka) byly sklizeny na počátku klimateria a uloženy do atmosfér s rozdílným obsahem CO_2 a O_2 po dobu 31 dnů při teplotě 3 °C. Vztah mezi složením plynné směsi v uvedené době a po jejím zrušení a uložením plodů do normálně kyslíkaté atmosféry byl vyjádřen k obsahu ethanolu, acetaldehydu, netěkavé sušině a texturním vlastnostem plodu. V anaerobních podmínkách dosahuje ethanol 1 109 mg/l pro odrůdu Valjevka a 628 mg/l pro odrůdu Stanleyova. Rozborem každého jednotlivého plodu se prokázal v anaerobních podmínkách konkávní vzestup ethanolu s jeho následujícím poklesem. Hladina kyslíku na úrovni 0.9 % kyslíku (ultranízký kyslík – ULO) není sice fyziologicky škodlivá, ale koncentrace ethanolu dosahují ve srovnání s anaerobními podmínkami asi poloviční hodnot. Uložení v atmosféře s normálním obsahem kyslíku trvalo do 53 nebo 68 dnů skladování, v němž se obsah ethanolu exponenciálně snižoval na hodnoty srovnatelné s plody uloženými trvale v atmosféře s dostatečným zásobením kyslíkem. Vzestup ethanolu u plodů skladovaných ve vzduchu v důsledku stárnutí měl exponenciální průběh po 20. dnu chladírenského skladování. Výsledná koncentrace po 53. dnu byla vyšší pro odrůdu Valjevka s hodnotou 828 mg/l než pro odrůdu Stanleyova s hodnotou 498 mg/l. Pevnost slupky byla rozdílná pro oba kultivary, rychlost měknutí byla vyšší u odrůdy Valjevka.

Klíčová slova: ethanol; acetaldehyd; nízký obsah kyslíku; švestky (*Prunus domestica* L.); pevnost

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