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Response of Controlled Atmosphere (CA) stored "Golden Delicious" Apples to the Treatments with Alcohols and Aldehydes as Aroma Precursors

Reaktion von CA-gelagerten „Golden Delicious" Äpfeln auf die Behandlung mit Alkoholen und Aldehyden als Aromavorstufen

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Summary

„Golden Delicious" apples were stored under ultra low oxygen (ULO-storage) and treated both at harvest time and after 5 months of storage with several aroma precursors. Another plot of fruits was sprayed on the tree with an ethylene inhibitor (AVG) to study the effect of ethylene biosynthesis on volatile production. ULO-storage caused a marked reduction on the ability of fruit to produce volatiles. Feeding these fruits with several alcohols and aldehydes as aroma precursors, stimulated the biosynthesis of volatiles. Each alcohol and aldehyde led to an enhanced production of the corresponding volatile, mainly esters. Similar to ULO-storage, AVG reduced the production of volatiles and this effect was accentuated during the storage of these fruits in ULO. Treating AVG-fruit with aroma precursors also led to a marked increase in the production of the corresponding volatiles. With ULO stored as well as with AVG-treated fruits, the effect of precursor treatment on volatiles was transient lasting, in general, not more than 2 days.

Zusammenfassung

„Golden Delicious" Äpfel wurden unter besonders niedrigen Sauerstoffkonzentrationen (ULO-Bedingungen) gelagert und sowohl bei der Ernte als auch nach 5 Monaten Lagerung mit verschiedenen Aromavorstufen behandelt. Ein weiterer Teil der Früchte wurde am Baum mit einem Ethyleninhibitor (AVG) gespritzt, um den Effekt der Ethylenbiosynthese auf die Aromabildung zu untersuchen. ULO-Lagerung verursachte eine deutliche Verminderung der Aromabildung der Früchte. Durch Behandlung solcher Früchte mit verschiedenen Alkoholen und Aldehyden als Aromavorstufen konnte die Biosynthese von flüchtigen Aromastoffen stimuliert werden. Jeder der verwendeten Alkohole und Ester verursachte eine gesteigerte Bildung von entsprechenden Aromastoffen, vor allem von Estern. Ähnlich wie die ULO-Lagerung verminderte die AVG-Behandlung ebenfalls die Bildung flüchtiger Aromastoffe, wobei diese Wirkung durch CA-Lagerung noch verstärkt wurde. Auch bei den mit Aromavorstufen behandelten AVG-Früchten erfolgte eine gesteigerte Bildung der entsprechenden Aromastoffe. Sowohl nach ULO-Lagerung wie auch nach AVG-Behandlung war durch die Behandlung mit Aromavorstufen nur eine vorübergehende Wirkung, im allgemeinen von nicht mehr als 2 Tagen, zu beobachten.

Introduction

Controlled atmosphere (CA) stored apples, especially those stored under ultra low oxygen (ULO), exhibit a diminished capacity to synthesize aroma volatiles. Although such apples remain firmer, juicer and greener, the lack of their ability to produce normal rates of volatiles led to critical acceptance by the consumers (BOHLING 1982, LEHMANN 1993). The reasons for this phenomena are still not completely understood. STREIF and BANGERTH (1988) proved that storage of apples at 1% O_2 reduced the biosynthesis of odor volatiles. YAHIA (1991) indicated that a post-storage period of 3 weeks at 3.3 °C after a low ethylene CA-storage did not improve the ability of apples to synthesize volatiles. It is supposed that the reduced biosynthesis of fatty acids, mainly of the unsaturated linolenic and linolic acids, is the main reason for the diminished odor volatile production (PAILLARD 1990), and this was confirmed by reasonable good correlations between the concentration of these fatty acids and aroma production (BRACKMANN et al. 1993).

The aim of this research was to investigate the effect of ULO-stored fruits on their ability to synthesize volatiles and whether the application of particular fatty acid-derived aroma precursors (alcohols, aldehydes, esters, and acids) can improve the biosynthesis of odor-volatiles. On the other hand, the relationship between ethylene biosynthesis and volatile production was tested throughout storage of "Golden Delicious" fruits treated with AVG on the tree to retard ethylene biosynthesis.

Material and Methods

The experiments were done in two consecutive years. "Golden Delicious" apple fruits were harvested in the orchards of the Experimental Station of Hohenheim University at Bavendorf, Germany. Fruits were brought

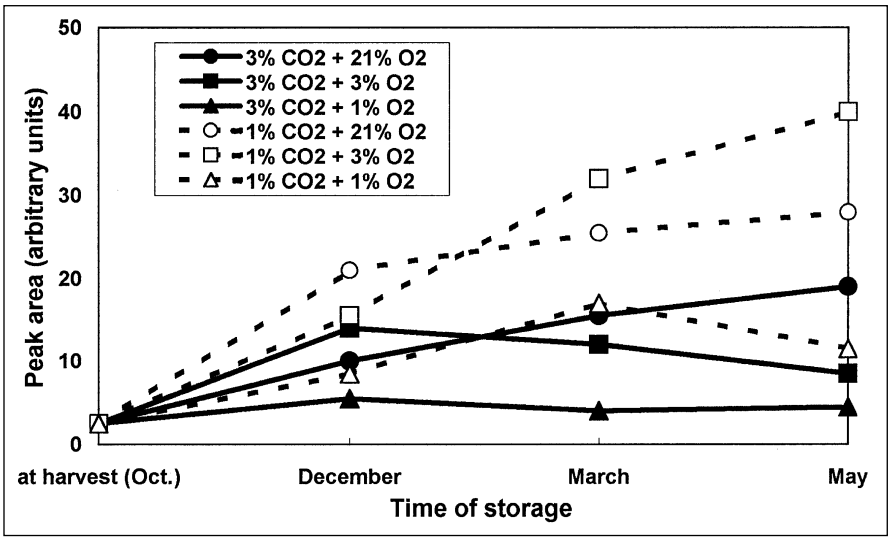


Fig. 1. The influence of different storage conditions on total aroma production of 'Golden Delicious' apples.
Einfluß der Lagerbedingungen auf die Gesamtaromabildung von "Golden Delicious" Äpfeln.

at the same day into ULO conditions at 3% CO_2 + 1% O_2; 1 °C and 93% R.H.. Directly after harvest and at two-months intervals during the subsequent storage period, aroma production was determined adopting the method of volatiles adsorption on small quantities of charcoal as described by STREIF (1981). In an other experiment 'Golden Delicious' trees were sprayed with aminoethoxyvinyl glycin (AVG), an inhibitor of ethylene biosynthesis, at a concentration of 200 mg/l. The trees were sprayed 4 times at weekly intervals starting one month before harvest. These fruits were also stored under the above conditions and subjected to the same treatments.

Directly after harvest and after 6-months of storage, several aroma precursors (see Table 2) were applied separately to the fruits. Within each treatment, 10 fruits were enclosed in 10 l desiccators and evacuated to 50-80 mbar. Each aroma precursor (0.1–0.15 ml) was injected into the evacuated desiccator, where it quickly evaporated. That creates a concentration of 5–7.5 ppm of each precursor. The pressure within the desiccator was released to ambient value within 25 min. Release of the partial vacuum caused a better and more uniform distribution of the precursors within the intercellular air spaces of the fruit. After treatment, the fruits were kept under 20 °C and flushed continually with normal air.

At the first and seventh day after the application of precursors, the volatiles of 50 l of the head-space air were adsorbed by inserting a Tenax AT column (60/80 mesh) into the outcoming air-stream of the continuously ventilated fruits. 25 ml diethyl ether plus 1 ml of an internal standard solution (500 ppm hexyl methylketon in pentane) were used to extract the adsorbed odor volatiles from the Tenax columns. Thereafter the final volume of the extract solution was reduced to 0.3 ml under N_2 gas stream. (A more detailed description of the method was published previously by SONG and BANGERTH 1996). 1 μl from this final extract solution was injected into a capillary GC: Detector: FID; column: 25m × 0.32 mm, ID fused silica CW 20M- DF-0.25; carrier gas: N_2; injector temperature: 240 °C; detector temperature: 260 °C; temperature program for the column: 3 min. at 35 °C, 35–100 at 5 °C/min., 100–180 at 6 °C/min., 10 min. at 180 °C.

A factor that represents the relative change of each odor-volatile was calculated based on the peak area of the standard. This factor was termed "r-factor". It comprises the peak area of a particular odor volatile after the

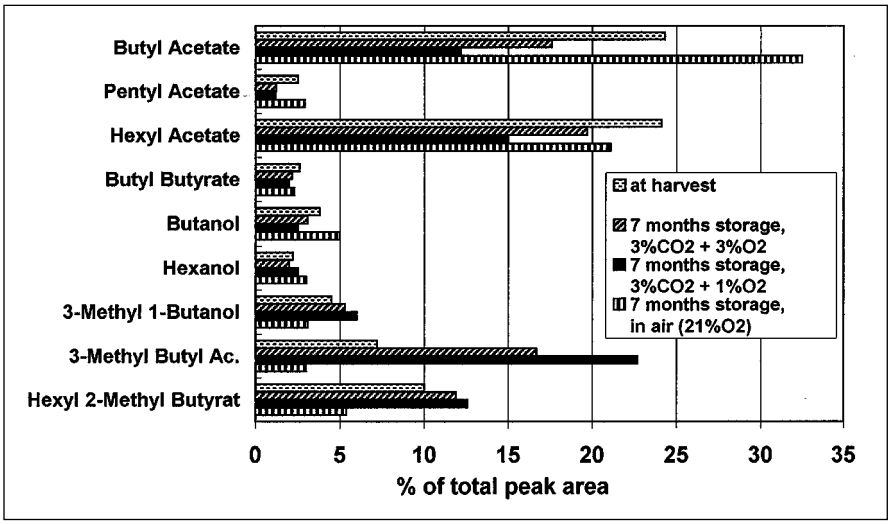


Fig. 2. Composition of aroma spectrum (%) of 'Golden Delicious' apples, directly after harvest (October) and after 7 months storage period (April) under different conditions.
Prozentuale Zusammensetzung des Aromas von „Golden Delicious" unmittelbar nach der Ernte (Oktober) und nach 7-monatiger Lagerung (April) bei unterschiedlichen Lagerbedingungen.

Table 1. Composition of aroma spectrum (arbitrary peak area units) of AVG-treated and control "Golden Delicious" apples, directly after harvest (October) and after 5 months in ULO-storage (March).
Zusammensetzung des Aroma-Spektrums (angegeben als Peakfläche-Einheiten) von „Golden Delicious" Äpfeln direkt nach der Ernte (Oktober) und nach 5 Monaten ULO-Lagerung (März) mit und ohne AVG-Behandlung.

	Peak Area Units			
	October		March	
Aroma components	AVG	Control	AVG	Control
Ethyl acetate	260	243	214	253
2-Propyl acetate	520	303	152	186
Propyl acetate	77	1 647	0	165
2-Methylpropyl acetate	0	207	0	245
Butyl acetate	811	40 219	113	9 218
Propyl butyrate	464	12 429	166	9 637
Butanol	157	2 882	0	491
Pentyl acetate	207	1 470	23	631
3-Methyl 1-butanol	0	638	25	473
Butyl butyrate	157	4 775	0	1 192
Butyl 2-methylbutyrate	71	4 216	0	2 290
Hexyl acetate	1 867	47 016	701	8 885
Pentyl butyrate	0	531	0	153
Hexanol	222	1 561	96	427
Hexyl2-methyl butyrate	1 529	16 374	1 160	4 596
n-Hexyl hexanoate	310	1 327	250	540
TOTAL	6 652	135 838	2 900	39 382

treatment divided by the peak area of the same odor-volatile emitted by the control fruits.

Table 2. The influence of feeding butanol to "Golden Delicious" apples, either directly after harvest (Oktober) or after 5 months under ULO-storage (March), on the synthesis of particular aroma components. Measurements were done 1 day after feeding and 7 days after feeding. Explanation for r-factor: see materials and methods.
Der Einfluss einer Begasung mit Butanol von "Golden Delicious" Äpfeln sofort nach der Ernte (October) oder nach 5 Monaten ULO-Lagerung (März) auf die Bildung einzelner Aromakomponenten. Die Messungen erfolgten einen Tag und sieben Tage nach der Begasung. Zur Erklärung des r-Faktors siehe Material und Methoden.

	r-Factor			
	October		March	
Aroma components	after 1 day	7 days	1 day	7 days
2-Propyl acetate	1.47	1,55	1,3	1,08
Ethyl propionate	0,32	0,52	0	
Propyl acetate	3,21	1,31	113,48	1,52
2-Methylpropyl acetate	0,55	0,94	1,01	1,19
Ethyl butyrate	41,77	19,58	2,71	1,47
Ethyl 2-methyl butyrate	24,33	0	1,1	1,45
Butyl acetate	2,32	1,01	13,24	1,29
Propyl butyrate	0,61	0,92	1,16	1,33
Butanol	3,46	1,15	11,2	1,5
Pentyl acetate	0,71	0,64	1,53	0,89
3-Methyl-1-butanol	0,78	0,99	0,97	1,25
Butyl butyrate	3,39	1,33	10,24	1,26
Butyl 2-methylbutyrate	2,01	1,23	6,76	1,66
Hexyl acetate	0,77	1,06	1,23	0,86
Pentyl butyrate	0,64	0,71	0,97	1,39
Hexanol	1,11	1,31	1,32	1,02
Hexyl 2-methylbutyrate	0,42	1,03	1,2	0,93
n-Hexyl hexanoate	1,81	0,51	1,15	0,62

Results

The influence of several storage conditions on the production of odor volatile is shown in Figure 1. It is noticed that reduced O_2 combined with increased CO_2 highly suppressed the ability of fruits to emit volatiles. After 7 months of ULO storage (3 % CO_2 + 1 % O_2), fruits synthesize only about 20 % volatiles compared to air stored apples. Figure 2 indicates the specific effect of ULO-storage on the particular aroma substances. It is seen that the synthesis of the quantitatively important aroma components: butyl acetate, pentyl acetate, hexyl acetate, and butyl butyrate was negatively influenced by ULO-storage, particularly at the most severe 3 : 1 conditions. However, on the other hand, the synthesis of the "branched" volatiles, 3-methyl butyl acetate, 3-methyl-1-butanol, and hexyl-2-methylbutyrate, was only slightly affected by this storage procedure.

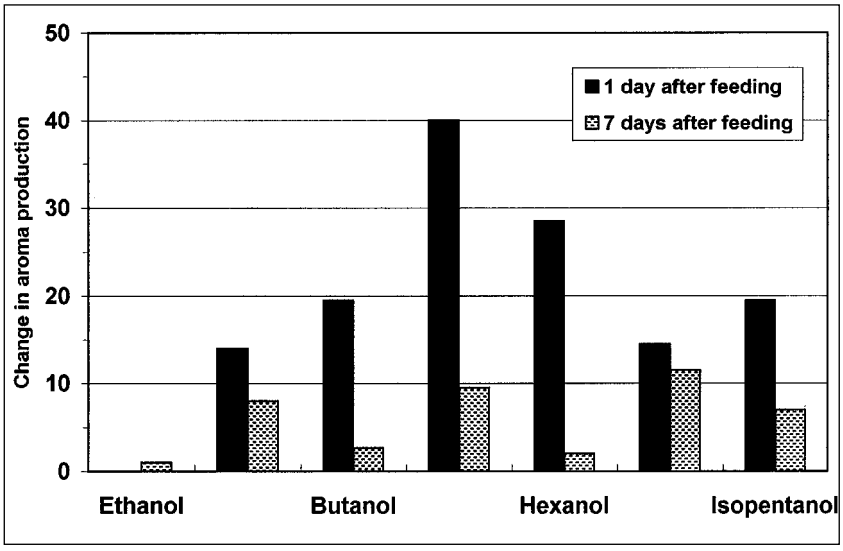


Fig. 3. Absolute change in the total aroma production of ULO-stored 'Golden Delicious' apples after feeding with different alcohol precursors; storage period was 6 months.
Veränderung (absolut) in der Gesamt-Aromabildung von „Golden Delicious" Äpfeln nach 6 Monaten ULO Lagerung und nach der Begasung mit verschiedenen Alkoholen.

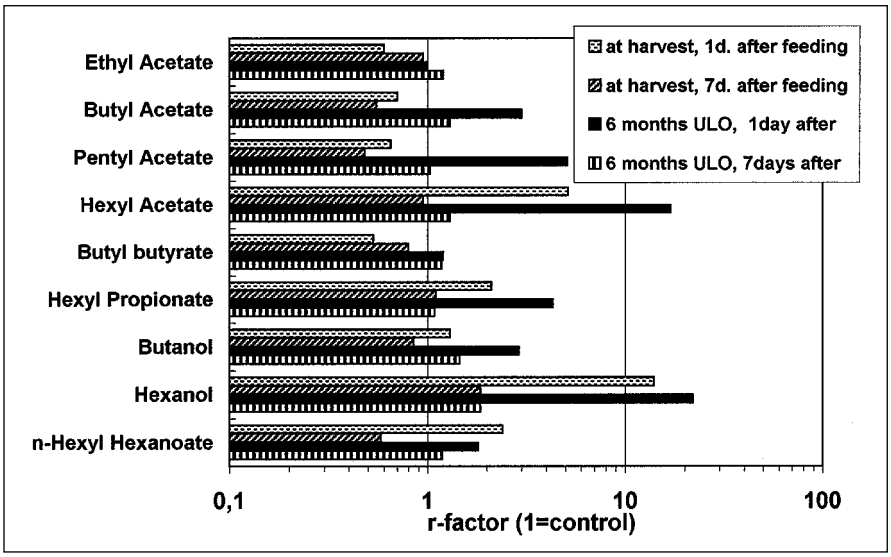


Fig. 4. The influence of feeding with hexanol to 'Golden Delicious' apples, either directly after harvest (October) or after 6 months (March) ULO-storage on the synthesis of particular aroma components.
Die Wirkung einer Begasung mit Hexanol entweder direkt nach der Ernte (Oktober) oder nach 6-monatiger ULO-Lagerung (März) auf die Bildung einzelner Aromastoffe bei „Golden Delicious" Äpfeln.

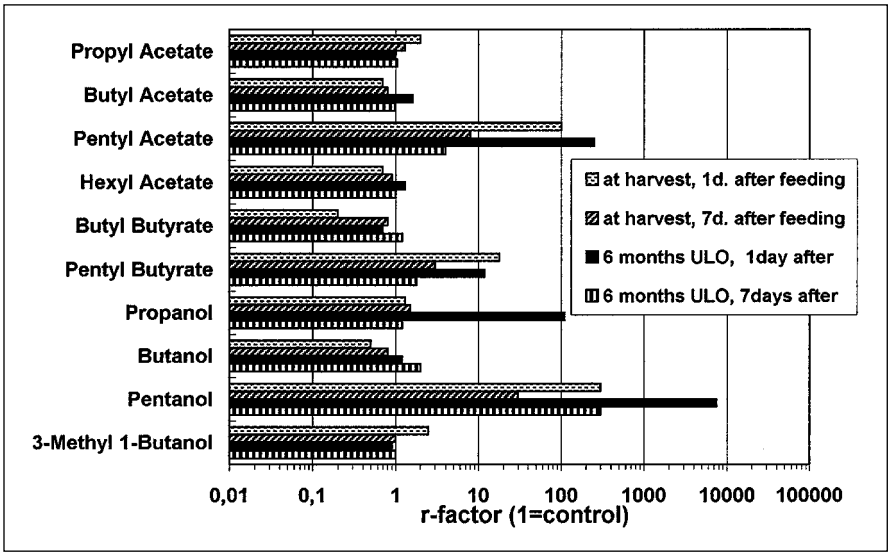


Fig. 5. The influence of feeding with pentanol to 'Golden Delicious' apples, either directly after harvest (October) or after 6 months (March) ULO-storage, on the synthesis of particular aroma components.
Die Wirkung einer Begasung mit Pentanol entweder direkt nach der Ernte (Oktober) oder nach 6-monatiger ULO-Lagerung (März) auf die Bildung einzelner Aromastoffe bei ‚Golden Delicious' Äpfeln.

Table 1 represents the effect of AVG-treatment on aroma production. It is noticed, that in contrast to ULO-storage the synthesis of the main volatiles, whether straight or branched chain volatiles, was highly diminished upon AVG-treatment. This AVG-effect persisted even after 5 months under ULO-storage conditions. It is also seen from the same table that both ULO-storage and AVG-treatment have had cumulative effects; in the sense that volatile production was almost eliminated when the AVG treated fruits were stored for 5 months in ULO-storage.

Results from Fig. 3 indicate the effect of feeding several alcohol precursors to fruits, that were stored for 5 months under ULO-conditions. The alcohols: propanol, butanol, pentanol, hexanol, isobutanol, and isopentanol considerably stimulated the synthesis of 'total odor' volatiles, measured 1 and 7 days after their applications. Table 2 shows the effect of feeding butanol on each particular odor volatile. Upon this treatment the synthesis of butyl acetate (the main odor volatile emitted by 'Golden Delicious' apple), butyl butyrate, butyl-2-methyl butyrate, and propyl acetate is considerably stimulated. On the other hand, feeding ULO-stored fruits with butanol leads to a reduced amount of hexyl acetate (20–30%). Hexyl acetate represents the second major odor volatile, which makes its reduction highly significant. It seems obvious that the increase in the biosynthesis of butyl acetate (130%) may have consumed to a significant extent the acetate, a common precursor for both hexyl- and butyl acetate. The feeding of freshly harvested or ULO-stored fruits with hexanol (Fig. 4) stimulated the biosynthesis of hexyl acetate, hexyl propionate, butyl butyrate, and butyl acetate to a greater extent for ULO-stored than for fruits just harvested. This is well documented, when the amount of hexyl acetate that is synthesized after treatment is compared for both dates.

The effect of a pentanol treatment, as the main odd numbered alcohol, was also investigated (Fig. 5). The feeding of apples with pentanol stimulated at both dates the biosynthesis of pentyl acetate, and pentyl butyrate. That leads, at the first date, to a slightly reduced biosynthesis of some other odor volatiles such as butyl acetate and butyl butyrate. The effect of the pentanol

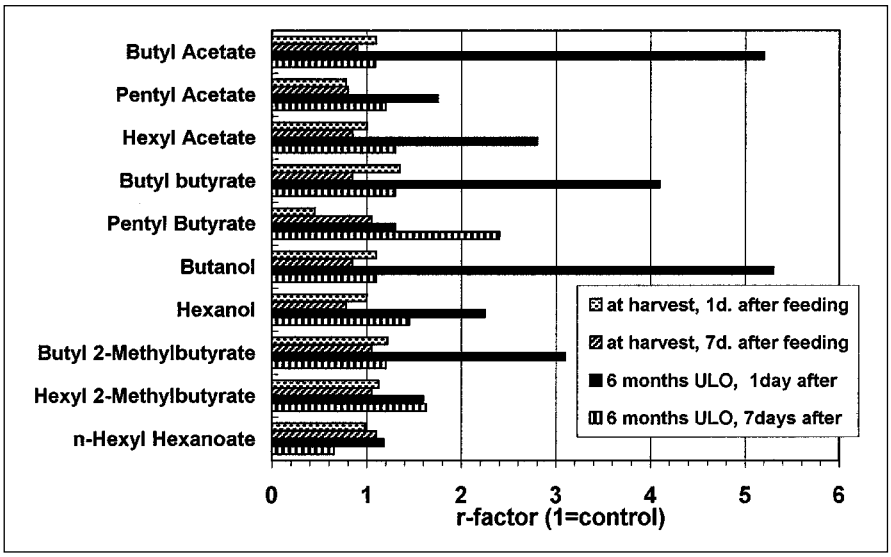


Fig. 6. The influence of feeding with butanol to 'Golden Delicious' apples, either directly after harvest (October) or after 6 months (March) ULO-storage on the synthesis of particular aroma components.
Die Wirkung einer Begasung mit Butanol entweder direkt nach der Ernte (Oktober) oder nach 6-monatiger ULO-Lagerung (März) auf die Bildung einzelner Aromastoffe bei „Golden Delicious" Äpfeln.

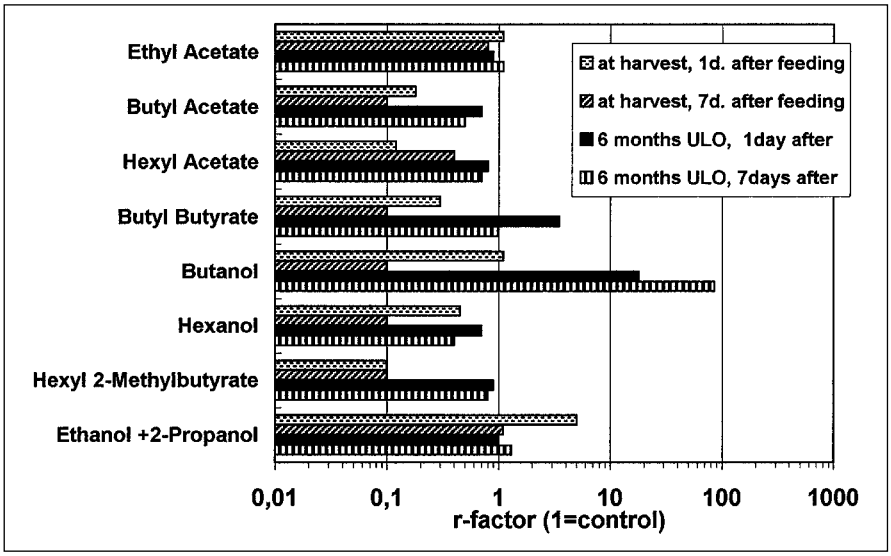


Fig. 7. The influence of feeding with butanol to AVG-treated 'Golden Delicious' apples, either directly after harvest (October) or after 6 months (March) ULO-storage on the synthesis of particular aroma components.
Die Wirkung einer Begasung mit Butanol entweder direkt nach der Ernte (Oktober) oder nach 6-monatiger ULO-Lagerung (März) auf die Bildung einzelner Aromastoffe bei „Golden Delicious" Äpfeln, die mit AVG behandelt waren.

treatment persist longer and stronger than that of other alcohols.

Concerning the effect of feeding the "branched" alcohol, isopentanol (3-methyl-l-butanol), table 3 shows that the biosynthesis of 3-methyl butyl acetate is strongly stimulated, an effect that still persists for more than 7 days.

The effect of feeding either freshly harvested or ULO-stored apples with aldehydes (e.g. butanal) is shown in Fig. 6. It is obvious that the stimulated biosynthesis of butyl acetate, butanol, butyl butyrate, butyl-2-methyl butyrate, hexyl acetate, and n-hexyl hexanoate was found in ULO stored fruits to last only for one day after feeding.

Fig. 7 shows the effect of feeding the alcohol precursor butanol to AVG-treated and ULO-stored fruits. It is clear from this treatment that feeding of these fruits with butanol caused an enhanced production of butyl acetate and butyl butyrate. However this enhanced effect persisted only for a short transient period. On the other hand, the feeding of these fruits with pentanol caused a marked increase in the production of several volatiles like pentyl acetate and pentyl butyrate (Fig. 8). The effect of pentanol persisted even for more than 7 days.

Discussion

ULO-storage results in a reduced biosynthesis of odor volatiles by 'Golden Delicious' apple fruit, where it affects mainly the biosynthesis of straight-chain volatiles, like butyl and hexyl acetate, while the biosynthesis of "branched" volatiles, mainly 3-methylbutyl acetate, was only slightly affected. On the other hand, it is well established that a pretreatment with the ethylene biosynthesis inhibitor AVG also reduced volatile production considerably, which confirms earlier results by HALDER-DOLL and BANGERTH (1987). In contrast to ULO-storage, however, AVG reduced stright- as well as branched-chain odor volatiles. This suggests that the "mode of action" of both treatments may not be identical.

The inhibitory effect of ULO-storage on volatile production could possibly be attributed to both lack of precursors for straight-chain volatile and ethylene bio-

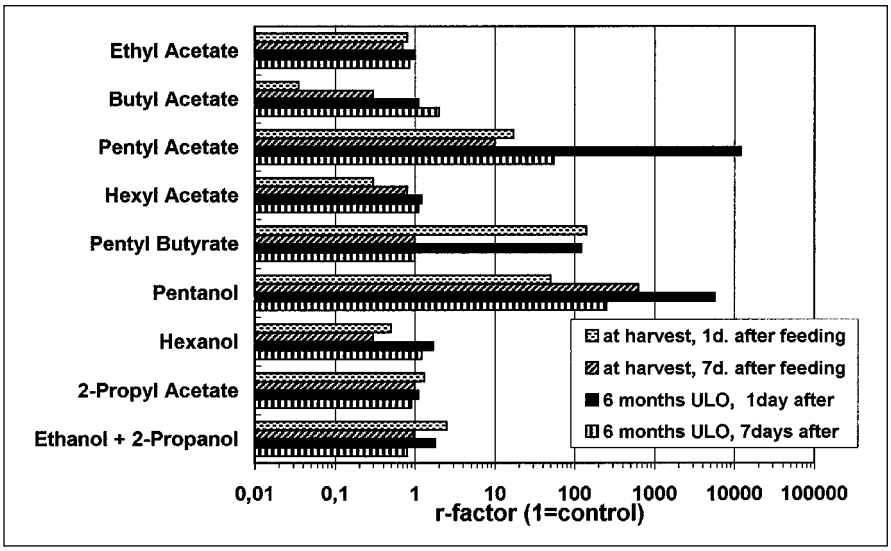


Fig. 8. The influence of feeding with pentanol to AVG-treated 'Golden Delicious' apples, either directly after harvest (October) or after 6 months (March) ULO-storage on the synthesis of particular aroma components
Die Wirkung einer Begasung mit Pentanol entweder direkt nach der Ernte (Oktober) oder nach 6-monatiger ULO-Lagerung (März) auf die Bildung einzelner Aromastoffe bei „Golden Delicious" Äpfeln, die mit AVG behandelt waren

synthesis and/or action. Both effects may be closely interrelated. Fatty acids, mainly the unsaturated ones, are precursors for these straight-chain volatiles (PAILLARD 1990) and one of their main oxidative metabolites, alcohols, are considered to be most important in volatile biosynthesis (DRAWERT et al. 1966). It is obvious from the results above, that feeding of fruits with alcohols highly stimulated the production of volatiles. The results indicate that a deficiency of alcohols, as precursors for volatiles, is one of the main reasons for the reduced biosynthesis of volatiles under ULO conditions as well as after AVG treatment. Alcohols react with organic acids, in an esterification process to produce esters (DRAWERT 1974, YAMASHITA et al. 1977). In agreement with the fact that alcohol precursors are oxidative metabolites of unsaturated fatty acids (STONE et al. 1975, GAILLARD et al. 1977), BRACKMANN et al. (1993) found, that the biosynthesis of these fatty acids was considerably reduced upon ULO-storage of "Golden Delicious" fruit. Similar and more detailed results will be presented by SONG and BANGERTH (in prep.). That lends support to the conclusion, that ULO-storage could affect volatile production via the biosynthesis of fatty acids, rather than being directly effective. Such an effect on fatty acid biosynthesis could occur at various stages in the fatty acids biosynthesis chain. The synthesis of acetyl CoA, the starter compound, could e.g. be inhibited through ULO-storage. The biosynthesis of acetyl CoA is localized in plastids (LIEDVOGEL 1985) and according to SLABAS et al. (1993) acetyl CoA should be firstly converted in the mitochondria to acetylcarnitine and than transported in this form to chloroplasts to be converted again to acetyl CoA. However, carnitine is synthesized through a methylation process, in which S-adenosyl-methionine (SAM) is essential (BENDER 1985). In this sense, it is possible that ULO-storage could create a deficiency in carnitine, since both respiration and ethylene biosynthesis are inhibited by ULO (STREIF and BANGERTH 1988).

Beyond that ULO-storage could affect fatty acid biosynthesis further through its effect on the acyl carrier protein (ACP), which represents the central enzyme in a multienzyme complex (OVERATH and STUMPF 1964, OHLROGGE et al. 1979). It was found before with apples and pears, that CA storage as well as AVG treatment can reduce protein synthesis as well as the activity of several ripening enzymes (SINGH et al. 1972, MELLENTHIN et al. 1980, HALDER-DOLL 1982). Depending on these facts, it seems possible that fatty acid biosynthesis is reduced due to a lack of adequate amounts of ACP, since the fatty acid biosynthesis is directly correlated with the content of ACP (OHLROGGE and KUO 1984). An additional point, where the biosynthesis of fatty acids could be affected is the process of fatty acid desaturation. This process is considered to be absolutely dependent on the partial pressure of O_2. (HARWOOD 1988) and requires considerable amounts of NADPH. Since the O_2 concentration under ULO-conditions is very low (1–2 %) it seems possible that the desaturation process will be partially inhibited by the lack of O_2 as well as by the low respiration of these fruit (STREIF and BANGERTH 1988) which will possibly not produce sufficient NADPH.

Another point that should receive more attention is the influence of the ripening hormone ethylene. It is obvious from our results, that AVG-treatment significantly reduced the production of volatiles, even more so than ULO-storage. From results shown above it is obvious that retarding ethylene biosynthesis will not reduce the activity of the esterification enzymes, since feeding of AVG-treated fruits with alcohol precursors, like pentanol, leds to an enhanced production of the corresponding volatile esters. The responsible esterases are, therefore, constitutively present and obviously independent of ethylene. Depending on these facts, it is probable that ethylene affects enzymes other than esterification enzymes, possibly those directly needed for fatty acids biosynthesis.

As another possibility ULO-storage as well as AVG treatment may reduce the sensitivity of the fruit for ethylene. BANGERTH (1984) claims that there is a reduction in tissue sensitivity possibly due to receptor alterations. Reasons for that could be the long and continuous absence of physiological ethylene concentrations (after AVG treatment) or the inability of the hormone to bind to its receptor due to the low O_2 concentration which finally leds to reduced synthesis. BLANKENSHIP and SISLER (1993) found that the number of binding

Table 3. The influence of feeding with 3-methyl-1-butanol to "Golden Delicious" apples, either directly after harvest (October) and after 5 months under ULO-storage (March), on the synthesis of particular aroma components. Measurements were done 1 day after feeding and 7 days after feeding. Explanation for r-factor: see materials and methods.
Der Einfluss einer Begasung mit 3-Methyl-1-Butanol von „Golden Delicious" Äpfeln sofort nach der Ernte (Oktober) oder nach 5 Monaten ULO-Lagerung (März) auf die Bildung einzelner Aromakomponenten. Die Messungen erfolgten einen Tag und sieben Tage nach der Begasung. Zur Erklärung des r-Faktors siehe Material und Methoden.

	r-Factor			
	October		March	
Aroma components	after 1 day	7 days	1 day	7 days
Ethyl acetate	0,5	0,95	0,95	1,11
2-Propyl acetate	0,78	1,34	1,04	1,31
2-Propanol+Ethanol	0,81	0	0,96	0,97
Ethyl propionate	0,66	2,14	0	0
Propyl acetate	1,1	0,8	43,54	0,89
2-Methylpropyl	0,65	0,96	0,67	0,97
1-Propanol	0,7	0,85	0	0,88
Butyl acetate	0,61	0,88	0,55	0,64
Propyl butyrate	5,58	2,28	10,81	3,05
Butanol	0,78	0,97	0,85	0,86
Pentyl acetate	0,79	0,92	3,6	0,76
3-Methyl-1-butanol	24,91	6,42	70,84	12,63
Butyl butyrate	0,76	1,02	0,7	0,6
Butyl 2-methylbutyrate	0,66	0,8	0,6	0,58
1-Pentanol	3,71	1,15	93,01	0
Hexyl acetate	0,75	0,99	0,91	0,82
Pentyl butyrate	1,63	1,08	1,37	0,74
Hexyl propionate	0,67	1,03	0,8	0,75
Hexanol	0,95	1,12	1,38	1
n-Hexyl hexanoate	3,19	2,52	0,84	0,53

sites in apple tissue did not differ during the preclimacteric or climacteric stages of apple fruit. Depending on these facts they suggest, that the affinity of binding sites for ethylene may differ instead. In this respect WHITEHEAD and BOSSE (1991) made the interesting observation that the sensitivity for ethylene of green banana fruit was improved through the application of short-chain fatty acids. Because of the reduced fatty acid biosynthesis after ULO-storage and AVG treatment it seems possible that also the affinity of the tissue for ethylene is affected in a manner that ethylene action is inhibited.

In conclusion, it seems that the main cause for the reduced aroma production of ULO-stored apples is its reduced ability to synthesize fatty acids. At present nothing is known, however, at what stage of fatty acid biosynthesis this impairment takes place.

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