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ALDEHYDE METABOLISM AND THE AROMA QUALITY OF STORED GOLDEN DELICIOUS APPLES

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Key Word Index—*Malus pumila*; Rosaceae; apple; aldehydes; carboxylic acids; controlled atmosphere storage.

Abstract—Aldehyde production by intact apples was monitored by reversed phase HPLC of headspace concentrates, after reaction with 2,4-dinitrophenylhydrazine. Depending on the degree of maturity and their storage history, Golden Delicious apples showed a variable headspace composition, differences being mostly of a quantitative nature. Whereas the headspace of pre-climacteric fruits was particularly rich in C_1–C_6 aldehydes, that of climacteric, ripening apples was greatly reduced, and some aldehydes were only present in trace amounts. Treatment of pre-climacteric or cold stored fruits with carboxylic acid vapours had a negligible effect on the aldehyde composition. Controlled atmospheric storage, however, led to a notable increase in the aldehydes derived from the added carboxylic acids or from those shortened by β-oxidation. This confirms the presence of a reductive path of carboxylic acids into aldehydes. Further results suggest that high carbon dioxide (CA-storage) interferes with carboxylic acid metabolism and alcohol dehydrogenase activity, leading to a deterioration of the aroma quality.

INTRODUCTION

Although aldehydes have been known to occur in fruits for a long time [1 and refs therein], the way they are synthesized is still only solved in part. It is established that hexanal and 3-hexenal are formed from linoleic and linolenic acids, respectively [2–5]. For the biosynthesis of C_3- to C_5-aldehydes, mostly circumstantial evidence points to the corresponding carboxylic acids at least in part as precursors. When apple discs [6] or intact apples [7, 8] are treated with carboxylic acids, an increase in the 'symmetrical' esters (e.g. propyl propionate) is observed. This indicates the sequence

$$RCOOH \rightarrow RCHO \rightarrow RCH_2OH \rightarrow RCOOCH_2R.$$

The first step is analogous to the reduction of palmitoyl- and stearoyl-CoA by *Euglena gracilis* and *Brassica oleracea* preparations [9, 10], or of substituted cinnamoyl-CoA thiol esters [11–13]. During experiments with intact apples which had been stored in controlled atmosphere cells (CA) application of propionic acid vapours not only led to a change in the volatile esters pattern, but also generated free propanal [7]. However, the headspace technique used in these experiments, by adsorption of the volatiles on Tenax GC, gives variable results for low boiling polar compounds. Thus it seemed worthwhile to repeat these investigations with the specific aim of studying aldehyde patterns, by applying a recently developed, more appropriate quantitation method [14].

RESULTS AND DISCUSSION

The headspace of Golden Delicious apples of different degrees of maturity and ripening usually contained the homologous series of C_1- to C_6-aldehydes in varying relative concentrations. Only in the case of pre-climacteric fruits were relatively high amounts of the aldehydes found with pentanal exceeding the other aldehydes (Tables 1 and 2, control; Fig. 1, control).

During post-harvest storing of the pre-climacteric apples at ambient temperature, no large changes occurred except in the case of pentanal, the concentration of which was almost reduced by half (Table 1, control).

When green, mature fruits were taken out of cold storage, their headspace showed low levels of aldehydes, which decreased slightly on further ripening (Table 2, control). If one accepts that carboxylic acids are precursors of aldehydes, the former observation may be correlated directly to the ripening phenomenon: at that moment large amounts of volatile esters are produced [8], the necessary alcohols for which are formed by reduction of the corresponding aldehydes [15]. If alcohols are removed almost as fast as they are formed, one might expect the aldehyde content to remain small provided that aldehyde synthesis is rate limiting. In order to test this supposition, both green and yellow mature Golden Delicious apples were treated with carboxylic acid vapours, and their aldehyde and ester production determined. The effect on the aldehyde concentration may be described as minimal (Table 2). Ester production on the other hand increased almost 10-fold (Table 3). This shows that aldehydes are transformed as quickly as they become available, and that in untreated, cold stored apples alcohol dehydrogenase and the ester forming enzyme system work at levels far below saturation.

Pre-climacteric apples (which form only small amounts, if any, of volatile esters, *cf.* [16]) did not react much on application of carboxylic acids (Table 1). This could mean that the aldehyde forming system works at full capacity or that the added carboxylic acids are not transformed into the activated form needed for reduction to aldehydes.

However, when Golden Delicious apples, stored in controlled atmosphere cells (CA stored), were treated with

Table 1. Aldehyde elimination by intact, pre-climacteric Golden Delicious apples after treatment with carboxylic acid vapours

Treatment	Days after treatment	Aldehydes (μg/kg/hr)						
		C_1	C_2	C_3	C_4	C_5	C^*_6	C_6
None (control)	1	1.9	4.9	1.0	4.0	64.3	0.6	
	3	1.6	1.4	0.5	1.7	12.3	0.7	—
	7	2.4	2.6	tr	3.7	26.2	2.7	—
	9	3.3	6.1	—	8.3	41.4	1.8	
	11	3.0	4.5	—	7.7	35.9	1.8	—
Acetic acid	1	2.4	15.7	0.9	8.1	38.2	2.1	
	3	3.0	1.5	—	0.6	5.2	—	—
	7	2.1	3.7	—	7.1	30.1	1.6	
	9	0.7	2.0		2.6	12.0	0.7	·
	11	2.9	5.7	—	8.8	46.9	1.5	—
Propionic acid	1	2.1	1.4	—	0.9	11.6	0.7	—
	3	2.0	3.5	—	1.9	22.3	1.1	—
	7	3.1	5.3	—	2.4	21.0	0.7	
	9	1.1	1.6	—	0.6	4.9	0.2	—
	11	3.0	4.1	—	4.3	28.1	1.1	—

*Hexenal

tr, <0.05 μg/kg/hr.

Table 2. Aldehyde elimination by cold stored Golden Delicious apples after treatment with carboxylic acid vapours, directly after removal from storage (green) and after a period of post-removal ripening of 11 days (yellow)

Treatment		Days after treatment	Aldehydes (μg/kg/hr)					
			C_1	C_2	C_3	C_4	C_5	C_6
None (control)	Green	1	0.3	0.3	0.1	0.3		—
		2	0.2	tr	0.1	tr	—	·
	Yellow	1	tr	0.1	tr	0.1	—	—
		2	0.1	0.2	tr	0.1		—
		4	0.3	0.2	0.1	0.3	0.1	tr
		7	tr	0.1	0.1	0.1	tr	tr
Acetic acid	Green	1	0.4	0.1	tr	0.1	0.3	—
		2	0.3	0.3	—	0.1	tr	tr
	Yellow	1	0.2	0.6	0.2	0.7	0.1	0.1
		2	0.3	0.4	0.2	0.4	0.1	tr
		4	0.9	0.4	0.2	0.9	0.1	0.1
		7	tr	0.1	tr	0.1	—	—
Propionic acid	Green	1	0.3	0.1	0.1	0.1	tr	—
		2	0.3	0.1	0.1	0.1	0.2	—
	Yellow	1	0.4	0.4	0.2	0.7	0.1	0.7
		2	0.3	0.4	0.2	0.5	0.2	0.1
		4	1.0	0.6	0.2	1.6	0.1	—
		7	0.6	0.4	0.1	0.6	tr	—
Butyric acid	Green	1	0.3	0.1	0.1	0.1	—	—
		2	0.1	tr	tr	0.1	—	—
	Yellow	1	0.1	0.2	0.1	0.4		
		2	0.1	0.2	0.1	0.3	tr	tr
		4	0.6	0.4	0.3	0.9	0.1	0.1
		7	0.1	0.2	0.1	0.2	tr	tr

tr, <0.05 μg/kg/hr.

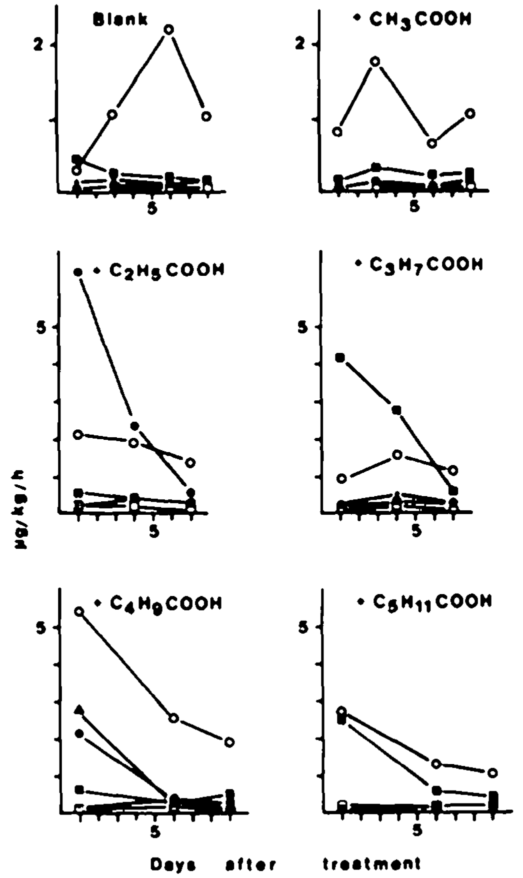

Fig. 1. Effect on aldehyde elimination by CA stored Golden Delicious apples of treatment with carboxylic acid vapours. Aldehydes: • —— •, C_1; O—O, C_2; ●——●, C_3; ■ —— ■, C_4; ▲——▲, C_5; □ ——□, C_6.

carboxylic acid vapours at ambient temperature, divergent behaviour was observed (Fig. 1). Application of acetic acid did not lead to a large change in the aldehyde pattern of the headspace. But with propionic, butyric, pentanoic and hexanoic acids noticeable increases of, respectively, propanal, butanal, pentanal plus propanal, and butanal occurred. This shows that carboxylic acids are precursors of aldehydes either directly or after β-oxidation. In tests with peel discs of CA-stored apples, the transformation of propionic acid into propanal still proceeded smoothly (blank: 1.6 µg/kg/hr; test: 28.2 µg/kg/hr).

From the results obtained with cold stored (Tables 2 and 3) and CA stored fruits (Fig. 1), it may be concluded at this point that both are able to produce aldehydes from exogenously supplied carboxylic acids. But whereas cold stored apples transform the aldehydes efficiently into volatile esters by way of the alcohols, CA stored fruits seem to be unable to handle increased concentrations of aldehydes. This is confirmed by the earlier finding that addition of propionic acid to CA stored apples leads to a change in the volatile ester pattern, but not to an extensively changed concentration [7]. The well documented diminished ester forming capacity of CA stored apples [16–19] could thus result from a change in the alcohol dehydrogenase activity in the fruit, leading to an alcohol shortage. This is supported by the work of Knee and Hatfield, who found that volatile ester formation is probably limited by the availability of alcohols [20]. The possibility of *in vivo* aroma enrichment of apples by treatment with alcohols was shown [21, 22].

In practice, the freshly harvested mature green Golden Delicious apples which are held in commercial stores are at the very start of the climacteric rise and are in fact thoroughly unripe. In order to obtain some information on the effect of carbon dioxide on the metabolism during CA storage, a batch of apples kept in cold storage for 2 months (analogous to the fruits used in the experiments of

Table 3. Volatile ester formation by cold-stored Golden Delicious apples during ripening, after application of carboxylic acid vapours

Volatile esters (µg/kg/15 min)	Control	Acetic acid	Propionic acid	Butyric acid
Propyl acetate	0.29	1.66	26.24	1.04
2-Methylpropyl acetate	2.14	7.23	8.55	3.67
Propyl propionate	—	—	66.63	—
Butyl acetate	23.35	109.30	60.08	83.68
3-Methylbutyl acetate	1.87	10.83	8.02	6.29
Propyl butyrate	—	—	13.46	—
Butyl propionate	0.40	4.09	68.77	2.90
Pentyl acetate	0.31	2.89	5.20	1.49
Butyl butyrate	1.71	32.03	15.58	129.29
Pentyl propionate	—	—	3.18	—
Hexyl acetate	3.90	58.32	45.70	20.65
Butyl 2-methylbutyrate	0.21	3.67	1.09	2.12
Propyl hexanoate	—	4.26	16.80	—
Hexyl propionate	0.19	1.99	42.89	0.67
Butylhexanoate + hexyl butyrate	2.70	47.47	23.99	54.81
Hexyl 2-methylbutyrate	0.48	5.57	2.10	1.40
Hexyl pentanoate	0.05	0.84	1.03	0.32
Hexyl hexanoate	0.74	10.73	5.48	2.28
Sum of the esters	38.34	300.88	414.79	310.61

Samples were taken from the ripe apples, Table 2, one day after application of the acids.

Table 4. Aldehyde elimination by Golden Delicious apples, first cold stored in air and then CA stored, on treatment with propionic acid vapour

Storage conditions	Treatment	Days after treatment	Aldehydes (μg/kg/hr)						
			C_1	C_2	C_3	C_4	C_5	C_7	C_9
Cold stored: 2 months	None (control)	1	3.1	61.5	tr	2.2	1.7	1.0	2.8
5% CO_2, cold: 6 weeks	Propionic acid	1	3.5	245.0	15.4	4.8	4.0	1.8	tr
Cold stored: 2 months	None (control)	1	3.6	40.4	tr	1.8	0.4	tr	tr
5% CO_2, cold: weeks	Propionic acid	1	1.3	52.2	2.6	0.8	0.1	tr	tr
Post-storage ripening at 20°: 1 week									

tr, < 0.05 μg/kg/hr.

Table 2), were transferred to 5% CO_2-air containers, and kept at 4° for 6 weeks. After this period, the aldehyde content of the headspace had changed noticeably: much higher concentrations were found than in the foregoing experiments, and heptanal and nonanal were present (Table 4). Treatment with propionic acid led once more to the accumulation of propanal, which did not occur to any large extent with cold stored fruits. The apples were then stored at 20° for a week under a continuous air flow. After this time they had started to yellow and to produce an aroma, and the aldehyde content of the headspace had diminished (Table 4). Addition of propionic acid still led to an increase of propanal, but to a much smaller degree than in the first experiment, which seems to indicate that the apples were recovering from the CA treatment.

In conclusion it may be stated that carboxylic acids are precursors of aldehydes, and that the antagonistic activity of carbon dioxide on the aroma formation might result not only from interference with alcohol dehydrogenase, but also (as deduced from the appearance of heptanal and nonanal in the headspace) with carboxylic acid metabolism.

EXPERIMENTAL

Fruits. Golden Delicious apples were obtained from the 'Veiling Produco' at St. Niklaas, Belgium (1982, 1983) (CA stored; 2% CO_2; 0 1°), from the test orchards at Meerdonk, Belgium (1982, 1983) and from a commercial grower at Zomergem, Belgium (1984, 1985) (cold stored; 0 1°).

Concentration and determination of headspace aldehydes. Apples (1.5 2 kg) were put in desiccators (± 8 l; 20°), which were continuously flushed with air (150 ml/min). The headspace was sampled by passing the eluting gases for 30 min through two scrubbers containing 2,4-dinitrophenylhydrazine reagent (6.5 ml of a soln of 125 mg 2,4-DNPH in 100 ml 6 M HCl, diluted with H_2O to 200 ml). The resulting hydrazones were isolated and quantified as described in ref. [14] in a Varian 8500 or a Varian 5000 liquid chromatograph. Treatment with carboxylic acids and analysis of the volatile esters were performed as described earlier [7, 8].

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