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Influence of water scrubbing on the production of volatile compounds and on sensory characteristics of 'Golden Delicious' apples stored in controlled atmosphere

P. Eccher Zerbini *, M. Grassi, A. Rizzolo

I.V.T.P.A.-Istituto Sperimentale per la Valorizzazione Tecnologica dei Prodotti Agricoli-via Venezian, 26-20133 Milano, Italy

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Abstract

The effect of the washing of the storage room atmosphere with water (water scrubbing) on the volatile production and on sensory quality of Golden Delicious apples (*Malus domestica* Borkh.) stored in controlled atmosphere (CA) was investigated. Atmospheres from control and water-scrubbed rooms were analyzed for ethylene and volatile compound composition over a 30-week storage period and, at the end of this period, the apples from the two storage modes were left to ripen at ambient temperature. The ready-to-eat ripe fruit were subjected to sensory analysis and their volatile composition was assessed by capillary gas chromatography.

Water scrubbing advanced the ripening processes in Golden Delicious apples: water-scrubbed fruit had more soluble solids, less titratable acidity, were less firm and more yellow. The differences between the fruit stored in the two rooms were perceived in the sensory analysis: water-scrubbed fruit were assessed sweeter and more aromatic than control fruit, while the latter were judged sourer and firmer. Water scrubbing did not reduce the concentration of ethylene and of other volatiles in the room atmosphere, but enhanced the production of selected compounds during storage (2/3-methylbutyl acetate, (E)-2-hexenol, hexyl acetate) and after poststorage ripening (pentyl acetate, butyl butanoate, octyl propanoate).

Keywords: Apples; Controlled atmosphere storage; Water scrubbing; Volatile; Quality; Sensory characteristics

1. Introduction

Among the techniques used to reduce ethylene and other volatile compounds in the storage rooms, the washing of the air with water (water scrubbing) is not widely used,

* Corresponding author. Fax: +39 (2) 236-5377.

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except for some apple cultivars in Italy and France, which are resistant to high relative humidity.

By water scrubbing the apple storage rooms, some benefits are achieved, such as less shrivelling and less weight loss (Gorini, 1990). These effects are mainly due to the increase in relative humidity (RH) in the cold room atmosphere; on the other hand, high RH can also increase other storage disorders, but by using controlled atmosphere (CA) these problems can be greatly reduced (Little and Barrand, 1989).

Both water scrubbing and CA influence the development of volatiles and flavour quality of fruit. According to Paillard (1966), water scrubbing caused a remarkable decrease in the amount of volatiles produced by cold stored Stayman apples, but did not affect the volatile composition.

CA storage reduces the production of the volatile compounds responsible for apple flavour. This suppressive effect depends on the composition of the storage atmosphere and on the length of storage, and it is particularly marked under ultra low oxygen (ULO) and high CO_2 conditions (Brackmann et al., 1993). Furthermore, CA influences the poststorage ripening of fruit at ambient temperature (Lidster et al., 1983; Patterson et al., 1974; Streif and Bangerth, 1988; Yahia, 1991). Even a little variation in the concentration of oxygen in CA can influence the volatile composition and flavour characteristics for Cox apples (Smith, 1984). The same phenomena were observed by Brackmann et al. (1993), Schamp and Dirinck (1982) and Willaert et al. (1983) for Golden Delicious apples.

The purpose of this study was to determine the effect of water scrubbing on ethylene concentration in the storage room, on volatile production and on quality of CA stored Golden Delicious apples.

2. Materials and methods

2.1. Fruit and storage

The research was carried out on Golden Delicious apples, harvested in one zone of Valtellina, cold-stored at 2°C in CA (1–2% O_2, 1–2% CO_2) in two identical commercial storage rooms of 1000 m^3, each filled with 250 tons of fruit of same maturity level. Each room was equipped with an active carbon scrubber, which was running for 5–6 h/d to remove excess CO_2. One of the rooms was fitted with a water tower (height 4.2 m, diameter 0.42 m), operating for 18–20 h/d with an air flow of 120 m^3/h and a water flow of 1.5 m^3/h (fresh water running to waste) over the whole storage time of 30 weeks. The two modes of storage are referred to as 'water-scrubbed' for the one with water scrubbing, and 'control' for the CA alone.

The rooms were closed until May. After 5, 9, 14, 17, 26 and 30 weeks of storage air was sampled for ethylene and volatile composition, and twenty fruits were taken out for quality analysis. This was done by first getting the fans circulating the atmosphere in each room, and consequently the air sample was taken. The fruit were sampled by opening the inspection window and taking out the apples that had already been prepared and placed nearby. The quality parameters measured on fruit were skin colour (Minolta Chromameter in reflectance), flesh firmness (11 mm diameter plunger), soluble solids

(SS) and titratable acidity. Then the fruit were divided into four groups of five units and each group was analyzed for sugar composition (sucrose, fructose, glucose) by high-performance liquid chromatography (HPLC) on a NH_2-column according to Forni et al. (1992).

At the end of the 30-week storage period, fruit from the two storage rooms were kept at ambient temperature (20°C) for 7 days for the poststorage ripening. Ready-to-eat ripe apples were subjected to sensory analysis. Portions of the flesh of the same fruit were used for the determination of volatile substance composition by static headspace.

2.2. Analysis of volatile substances

Ethylene in storage atmosphere

Two litre gas samples from storage atmospheres were collected into Tedlar gas sampling bags by using a personal air sampling pump. The gas samples were assayed by a gas chromatograph (DANI, model 3800, Monza, Italy) equipped with a 3-ml loop injection valve, a 2-m stainless steel Alumina F1 (80–100 mesh) column held at 100°C, and a flame ionization detector at 250°C. Data were expressed as ppm (μl/l). The analysis after 30 weeks failed.

Volatiles in storage atmosphere

The volatile compounds of 50 litres of refrigerated atmosphere were adsorbed on 200 mg of Carbotrap (20–40 mesh) by flowing the air through a tube connected to a personal air sampling pump preset at a flow rate of 1 l/min. Then the desorption of the samples from the sorbents was achieved using the thermal desorber TD 120b (DANI) fitted onto the injector port of the gas-chromatograph (170°C for 20 min; injection time 5 s). Gas chromatographic separations were achieved using a Carbowax 20 M column (25 m × 0.5 mm i.d., 1 μm film thickness) according to the conditions reported by Rizzolo et al. (1992). The analysis of water-scrubbed room after 26 weeks failed

Volatiles in fruit

One gram of peeled and minced fruit flesh was put in a sealed 4.5-ml vial and conditioned at 70°C for 30 min; a 1-ml gas sample was withdrawn from the headspace gas using a gas-tight syringe and analyzed by gas-chromatography on a Carbowax 20 M column (25 m × 0.5 mm, 1 μm film thickness) according to Rizzolo et al. (1992). Each sample was obtained by pooling a slice per fruit used for the sensory analyses and mincing the whole.

Volatile identification and quantification

The identification of volatile compounds was performed according to their retention times, comparing them with those of commercial standards and/or with gas-chromatography/mass spectrometric data obtained for a ripe Golden Delicious aroma (Rizzolo et al., 1992).

The amounts of the identified compounds were estimated by relating the peak area of each compound to that of the corresponding standard, and were expressed as μmol per g of fruit flesh or μmol × 10^{-6} per l of air.

The unknown 1 and 2 compounds, butyl propanoate, butyl butanoate, (E)-2-hexenyl acetate, hexyl butanoate + hexyl 2-methyl propanoate, octyl propanoate + 2-ethyl hexanol, hexyl hexanoate and (E)-2-hexanol were estimated as μmol per g of fruit flesh or μmol $\times$ 10^{-6} per l of air of hexyl acetate.

2.3. Sensory analysis

For sensory tests, fruit from the two rooms were peeled, sliced and presented to a taste panel of twelve people; six panellists were IVTPA staff, with some experience in taste testing, and six were students, without experience. The triangle test was used (Meilgaard et al., 1987), replicated on seven occasions during the experiment. Each sample was identified by a random three digit code. The position of the samples within the triangle was randomized. The sensory test was carried out in a panel room with separate booths for each panellist.

Judges were asked three questions: (1) to identify the odd sample, (2) to indicate how the odd sample differed from the duplicate ones, and the direction of the difference (i.e. 'more' or 'less'), (3) to point out the preferred sample. The answers to the last two questions were accepted only if the odd sample had been correctly identified.

2.4. Statistical evaluation

Volatiles in the storage atmospheres: having only one replication at each time of analysis, the volatile composition of the storage atmospheres was evaluated to see if there were consistent differences between the two rooms or a consistent trend of accumulation or dilution of the different compounds; so, data for single compounds were processed by a General Linear Model procedure (SAS GLM) considering storage room as a classification variable and weeks of storage as a continuous variable, with a linear or a quadratic effect.

Volatiles and quality variables of fruit were processed by analysis of variance.

Triangle tests: results were evaluated according to O'Mahony (1986). Answers to question (1) for each judge were evaluated by one-tailed binomial test with $P = 1/3$; answers to questions (2) and (3) were evaluated by two-tailed binomial test with $P = 1/2$ (O'Mahony, 1986, Table G.4.c and Table G.4.b).

3. Results

3.1. Quality of fruit during storage

During storage, in the water-scrubbed fruit SS content was higher, titratable acidity and firmness was lower; the latter decreased more steeply than in control fruit (Table 1). Colour shift from green-yellow to yellow was the same in the two storage rooms up to 17 weeks of storage; afterwards the water-scrubbed fruit became more yellow, while control fruit did not change further until the end of storage: hue values of control fruit at 30 weeks were not different from those of water-scrubbed fruit at 17 weeks.

Water-scrubbed fruit showed a maximum content of fructose, glucose and total sugars after 26 weeks of storage; in control fruit glucose increased during storage and fructose

Table 1
Means and F-ratios of firmness, hue, soluble solids (SS) and titratable acidity in water-scrubbed (WS) and control apples during the storage period (20 replications)

Storage time (weeks)	Means							
	Firmness (N)		Hue (radians)		SS (°Brix)		Acidity (meq/100 g)	
	control	WS	control	WS	control	WS	control	WS
9	63.2	58.1	1.980	1.961	15.61	16.43	6.94	6.46
14	61.5	57.8	1.789	1.789	15.58	15.81	6.10	6.46
17	59.7	54.2	1.825	1.738	14.72	15.94	6.27	5.92
26	60.9	52.5	1.805	1.707	14.93	16.23	5.56	5.55
30	61.3	48.6	1.726	1.673	15.46	15.82	5.41	4.86
SEM[a]	1.395		0.016		0.207		0.156	

Source of variation	Degrees of freedom	F-ratio			
		Firmness	Hue	SS	Acidity
Time	4	5.52 **[b]	80.83 **	2.91 *	29.16 **
Storage treatment	1	63.86 **	24.12 **	36.26 **	9.02 **
Storage × time	4	3.15 *	3.85 **	2.78 *	1.16

[a] Standard error of the mean.
[b] * indicates significant for $P \geq 95\%$; ** indicates significant for $P \geq 99\%$.

showed a maximum after 17 weeks of storage. In fruit from both storage rooms sucrose decreased with time of storage (Table 2). The trends of sucrose and glucose during storage agreed with those found for Golden Delicious and other apple cultivars (Eccher Zerbini et al., 1979; Hulme, 1958).

3.2. Sensory analysis

In sensory tests only three judges, all experienced panellists, were able to distinguish the fruit from the two storage modes, recognizing correctly the odd sample in 5 or more triangle tests out of seven ($P < 0.05$); the remaining 9 judges gave a lower number of correct answers, not different from the chance level. A total of 42 tests out of 84 were correctly answered. Preferences were equally distributed between the treatments.

Table 3 shows the attributes given by all the tasters in the correctly answered tests. Most of the attributes given by tasters indicated that water-scrubbed fruit tasted riper than control fruit; this can be considered significant only for sourness, sweetness and firmness, and near the limits of significance ($P = 0.06$) for the intensity of aroma: water-scrubbed fruit were assessed sweeter and more aromatic than control fruit, while the latter were judged sourer and firmer.

3.3. Volatiles in fruit after storage

Table 4 shows the headspace volatile composition of control and water-scrubbed fruit after 30 weeks of storage and seven days at 20°C.

Table 2
Means and F-ratios of sugars (g/100 g FW) in water-scrubbed (WS) and control apples during the storage period (4 replications)

Storage time (weeks)	Means							
	Glucose		Fructose		Sucrose		Total	
	control	WS	control	WS	control	WS	control	WS
9	1.87	2.24	8.34	8.71	3.11	3.38	13.32	14.32
14	2.96	3.33	8.30	8.60	3.82	2.65	15.08	14.58
17	3.34	3.82	9.57	8.41	1.82	2.53	14.73	14.75
26	3.66	4.67	8.24	10.72	1.84	2.44	13.74	17.82
30	3.62	2.72	8.24	8.36	2.39	2.56	14.26	13.63
SEM [a]	0.274		0.529		0.329		0.717	

Source of variation	Degrees of freedom	F-ratio			
		Glucose	Fructose	Sucrose	Total
Time	4	15.77 ** [b]	1.66	5.55 **	2.43
Storage treatment	1	2.28	1.54	0.29	3.03
Storage × time	4	3.30 *	3.05 *	2.59	3.69 *

[a] Standard error of the mean.
[b] * indicates significant for $P \geq 95\%$; ** indicates significant for $P \geq 99\%$.

Table 3
Sensory analysis of apples after storage in control and water-scrubbed (WS) rooms: attributes by which the samples were perceived to differ, number of times each one was mentioned and direction of the difference. Attributes were collected only when the panellist identified correctly the odd sample; in each test more than one attribute could be given

Attribute	Number of times mentioned	Direction of the difference		P [a]
		Control > WS	WS > Control	
Sour	15	12	3	0.035
Sweet	28	8	20	0.036
Aromatic	19	5	14	0.064
Firm	20	17	3	0.003
Juicy	14	9	5	
Mealy	2	-	2	
Overripe	2	-	2	
Ripe	1	-	1	
Astringent	1	1	-	

[a] Probability of obtaining such a majority.

The main compounds emitted after storage by Golden Delicious apples (i.e. those in amounts above 10 μmol × 10^{-4}/g) were acetaldehyde, ethanol, butanol, octyl propanoate +2-ethyl hexanol and pentyl acetate. (E)-2-hexenal was not detected.

Fruit showed a great variability in the composition of volatiles; nevertheless, some differences were significant: water-scrubbed fruit had higher amounts of octyl propanoate + 2-ethyl-hexanol, butyl butanoate, pentyl acetate and unknown 2.

Table 4
Headspace volatiles (μmol × 10^{-4}/g) in fruit after storage (6 replications). Means for storage room are reported only if significantly different. WS = water-scrubbed

Compound	Storage room		Mean	SEM [a]
	Control	WS		
Unknown 1 [b]			2.491	0.182
Acetaldehyde			204.442	42.330
Acetone			1.548	0.288
Unknown 2 [b]	0.444	0.813	0.628	0.089 *
Ethyl acetate			2.648	0.643
Butyl acetate			4.445	1.263
Ethanol			104.729	19.547
Ethyl propanoate			1.805	0.382
2/3-methylbutyl acetate			0.583	0.326
Ethyl butanoate			2.290	0.459
Ethyl 2-methyl butanoate			0.059	0.021
Hexanal			0.078	0.052
Butanol			34.464	6.091
Butyl propanoate [b]			6.109	1.837
Pentyl acetate	6.316	18.178	12.243	2.962 * [c]
(E)-2-hexenal			0.00	0.00
Butyl butanoate [b]	1.848	6.234	4.041	0.950 *
Hexyl acetate			1.758	0.738
(E)-2-hexenyl acetate [b]			0.099	0.099
Hexanol			6.529	0.924
Hexyl butanoate + hexyl 2-methyl butanoate [b]			2.875	0.918
Octyl propanoate + 2-ethyl hexanol [b]	8.455	47.874	28.164	8.176 **
Hexyl hexanoate [b]			3.097	0.458
(E)-2-hexenol [b]			0.841	0.751

[a] Standard error of the mean.
[b] Expressed as μmol × 10^{-4}/g of hexyl acetate.
[c] * indicates a difference between rooms significant with $P \geq 95\%$; ** indicates a difference between rooms significant with $P \geq 99\%$.

3.4. Volatiles in storage atmosphere

Quadratic effect of time was generally higher than linear effect, except for (E)-2-hexanol and total volatiles (Table 5). Significant interactions of time with storage atmosphere were found for ethyl propanoate, 2/3-methylbutyl acetate and hexyl acetate: in the last months of storage ethyl propanoate increased in the control room and disappeared in the water-scrubbed room; on the contrary, 2/3-methylbutyl acetate was not found after 30 weeks of storage in control room, and it was detected in relatively high amounts in water-scrubbed room; hexyl acetate increased steadily in control room while it was very high only after 30 weeks of storage in water-scrubbed room.

Ethyl butanoate, (E)-2-hexenal and butyl butanoate increased in the last months of storage in both rooms. The unknown 2 disappeared after 26 weeks of storage, and it was

Table 5
Volatile composition (μmol × 10^{-6}/l) of cold room atmospheres during the storage period. Means are reported only for significant effects

Compound	Storage room	Weeks of storage						Means for rooms	General mean	SEM[b]	F-ratio		
		5	9	14	17	26	30				Storage room	Time²	Room × time²
Unknown 1[a]									0.243	0.053	0.09	0.83	1.88
Acetaldehyde									10.011	3.386	0.75	0.28	1.02
Acetone									1.311	0.352	0.03	0.34	0.12
Unknown 2[a]	mean	0.85	0.64	0.40	0.75	0.00	0.00	control 0.73 WS[c] 0.19	0.484	0.103	9.52**[d]	8.61 **	2.12
Ethyl acetate									1.782	0.521	0.59	0.44	0.08
Butyl acetate									0.755	0.231	2.28	2.09	3.09
Ethanol									3.913	1.630	0.20	3.13	0.04
Ethyl propanoate	control	0.76	1.02	0.80	1.80	3.02	2.24		1.193	0.155	0.44	0.72	13.27 **
	WS	1.08	1.20	1.20	0.00	-	0.00						
2/3-methylbutyl acetate	control	0.58	1.38	1.64	0.82	0.16	0.00		1.300	0.146	1.05	6.21 *	30.62 **
	WS	1.56	0.60	1.42	1.90	-	4.24						
Ethyl butanoate	mean	8.01	13.66	4.05	23.69	16.52	71.00		23.395	4.195	0.00	26.34 **	2.78
Ethyl 2-methyl butanoate									0.062	0.042	0.01	1.78	0.02
Hexanal									11.084	2.698	0.31	1.68	0.03
Butanol									28.380	7.489	0.02	1.22	0.43
Butyl propanoate[a]									8.045	1.725	0.04	0.38	0.01
Pentyl acetate									9.120	1.572	1.47	0.08	3.91
(E)-2-hexenal	mean	1.65	9.58	2.58	8.46	30.12	21.47		10.691	2.206	0.00	12.97 **	0.19
Butyl butanoate[a]	mean	2.67	3.21	1.57	2.32	11.18	12.87		5.136	0.797	1.00	29.04 **	3.38
Hexyl acetate	control	1.14	0.80	2.68	5.48	10.7	9.98		6.324	0.706	4.09	119.56 **	31.57 **
	WS	0.42	1.46	0.94	4.56	-	31.4						
(E)-2-hexenyl acetate[a]									1.265	0.582	0.36	0.05	0.90
Hexanol									9.855	2.161	0.29	0.21	0.25
Hexyl butanoate + hexyl 2-methyl propanoate[a]									4.287	2.208	0.05	2.92	0.01
Octyl propanoate + 2-ethyl hexanol[a]	mean	0.36	0.26	0.26	0.00	0.00	1.25		0.390	0.109	0.95	9.78 **	4.04
Hexyl hexanoate[a]									4.180	3.823	0.01	0.68	0.78
(E)-2-hexenol[a]								control 0.073 WS 0.532	0.282	0.118	5.92 *	4.07[e]	3.32[f]
Ethylene (ppm)	mean	60.75	67.15	68.00	67.25	109.85	-		67.8	4.14	0.28	37.58 **	0.67
Total volatiles	mean	103.3	118.2	119.4	153.3	224.4	182.8		143.5	11.90	0.69	9.23 *[e]	1.69[f]

[a] Expressed as μmol × 10^{-6}/l of hexyl acetate.
[b] Standard error of the mean
[c] WS = water-scrubbed
[d] * indicates significant for $P \geq 95\%$; ** indicates significant for $P \geq 99\%$.
[e] Linear effect of time.
[f] Linear interaction of room × time.

higher in the control room. (E)-2-hexenol was higher in water-scrubbed atmosphere.

Ethylene concentration was not different in the two storage modes and it was highest at 26 weeks of storage.

Total volatile compounds increased during storage in both rooms.

4. Discussion

The fruit in the two storage rooms were similar at the beginning of storage; the quality of fruit stored in the control room did not change remarkably during storage, while the fruit stored in water-scrubbed room showed a yellowing of the skin, and a reduction of acidity and of firmness; as the storage progressed, so these differences became greater.

As for the volatiles detected in the headspace of ripe fruit, the only difference between the two rooms was a higher amount of butyl butanoate, pentyl acetate, and octyl propanoate in water-scrubbed fruit; these esters are present in great amounts just before the beginning of fruit senescence (Paillard and Matteì, 1973).

These results were confirmed by sensory analysis: water-scrubbed fruit were assessed sweeter and more aromatic, less sour and less firm than control fruit.

Considering these results, it would seem that the water scrubbing induces earlier ripening of fruit, compared to the control; yet it is known that ripening is generally associated with increasing ethylene production. Actually, water scrubbing did not influence the concentration of ethylene, nor the trend of total volatiles. Ethylene concentration in model atmospheres with standard gas mixtures was slowly reduced by water scrubbing (Polesello et al., 1986); Duvekot (1961) found that the atmosphere of water-scrubbed storage room had milder apple flavour than the control room.

The volatile composition of the storage atmospheres cannot be considered as the direct effect of the volatile production of fruit under storage, as CA storage rooms are not closed systems. Obviously, all the equipment used to maintain atmosphere and temperature could cause adsorption, diffusion or dilution of volatile compounds developed by stored fruit. Our experiment was carried out in two identical rooms, with two separate and equal equipment for scrubbing carbon dioxide; so, presumably, there should have been a greater loss of volatiles in the water scrubbed atmosphere, either for the mechanical effect or for the solubility in water. Among the volatiles found in the atmosphere, only ethyl propanoate and the unknown 2 were lower in water-scrubbed room; some authors have found that ethyl propanoate tended to increase during the early stages of cold storage and then declined to very low levels (Chieraghi-Seifabad et al., 1989; Forsyth et al., 1969; Lidster et al., 1983). Other compounds, even if soluble in water, like 2/3-methylbutyl acetate and (E)-2-hexenol were present in higher amounts in water-scrubbed room, as well as hexyl acetate, which is insoluble in water; the higher concentration of the latter compounds could be due to an enhanced production by water-scrubbed fruit. Esters are produced in higher amounts by ripe Golden Delicious fruit (Dirinck et al., 1989): all these findings suggest that the pattern of volatiles found in the atmosphere of water-scrubbed room is related to riper fruit.

The causes of earlier ripening of water-scrubbed fruit are not clear. Water scrubbing is known to influence relative humidity (RH). In this experiment RH in storage rooms was not measured, but Duvekot (1961) found higher RH in water-scrubbed room (93–97% with a water tower, as compared to 87–93% in normal storage); we can presume that the countercurrent flow of air in water could have increased RH in the atmosphere. It could be envisaged that water scrubbing actually removed part of the volatiles, including ethylene, which could have been counterbalanced by a greater production by the fruit. So, ripening could be the response of fruit to this dynamic situation (higher RH, scrubbing of volatiles) caused by water scrubbing.

5. Conclusions

Water scrubbing advanced the ripening processes in Golden Delicious apples; in fact apples stored in water-scrubbed atmosphere had more soluble solids, less titratable acidity, were less firm and more yellow. The differences between the fruit stored in the two rooms were perceived in the sensory analysis: water-scrubbed apples were considered sweeter, more aromatic, less sour and firm than control fruit. These characteristics are well appreciated by Italian consumers.

Water scrubbing did not reduce the concentration of ethylene and of other volatiles in the room atmosphere, but enhanced the production of some esters during storage and after poststorage ripening.

As apples stored for a long period in CA often suffer from lack of flavour, so the changes in volatile composition achieved by using water scrubbing could be an interesting feature.

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