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Effect of Several Post-Harvest Fungicide Treatments on Carbohydrate Evolution of Cold Stored Apples

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ABSTRACT

Post-harvest fungicide treatments were used to preserve 'Starking' and 'Golden Delicious' apples during cold storage. The fungicide compounds utilized in the experiment were benomyl, carbendazim, imazalil, thiophanate-methyl and thiabendazole. Treatments were by immersion of some fruits into fungicide aqueous solutions at recommended concentrations and immersion times.

Determination of the most important carbohydrates in apples (sucrose, fructose, glucose as well as sorbitol) was by high performance liquid chromatography.

Post-harvest fungicide treatments have caused an increase in carbohydrate content of fruits relative to the untreated apples of the same cultivar and an important improvement in their ripening. Apples treated with imazalil showed greater dehydration and excessive wrinkling of fruit peel.

INTRODUCTION

The large production of apples in most countries makes necessary a new storage technology to preserve the fruits from decay caused by post-harvest diseases. Losses of apples during their cold storage were about 30% of world production (Green, 1983). The use of post-harvest chemical treatments helps to extend the storage time of fruits (Eckert, 1977) but little is known about the effects of these chemical compounds, applied after harvest and before storage, on the quality of treated fruits. Few papers have reported the

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influence of pesticides on quality attributes of fruits during the growing-season (Taylor & Mitchell, 1956; Riehl *et al.*, 1957; Dean & Bailey, 1960; De la Plaza & Alique, 1984).

High performance liquid chromatography has been successfully used to determine sugars in a number of fruits and vegetables, providing accurate and reliable results. In the present work the influence of various chemical treatments (benomyl, carbendazim, imazalil, thiophanate-methyl and thiabendazole) on the carbohydrate content of 'Starking' and 'Golden Delicious' apples during cold storage is investigated.

MATERIALS AND METHODS

Apples

Two cultivars of apples were used for experiments, 'Starking' and 'Golden Delicious'. The apples were grown under commercial conditions at the 'Cortijo del Río' ranch near Antequera (Málaga, Spain). After harvest, the apples were transported to the Food Processing Pilot Plant at the Instituto del Frío (CSIC), Madrid. Then fruits were selected according to their size and stage of maturity. Only apples free of injury were treated.

Fungicide treatments

Twenty-four hours after harvest, treatments were carried out by immersion of fruits in aqueous solutions of each fungicide compound at the concentrations and times of immersion shown in Table 1. Fruits were then placed in open plastic boxes and stored in 85–90% of relative humidity and at 0°C (cv. 'Starking') or +2°C (cv. 'Golden Delicious').

TABLE 1
Post-harvest Fungicide Treatments Applied to Apples

Fungicide	*Commercial name*	*Concentration*[a] *(μg/ml)*	*Immersion times*[a]
Benomyl	Benlate	500	1 min
Carbendazim	Bavistin	500	1 min
Thiophanate-methyl	Pelt	1 000	1 min
Thiabendazole	Tecta 60	1 000	35 s
Imazalil	Deccozil-S-7·5	1 000	1 min

[a] Concentrations and immersion times were found in the related literature for use of these compounds on cold stored pome fruits.

Analysis of carbohydrates

Sucrose, fructose, glucose and sorbitol were measured by the high performance liquid chromatographic method reported by Alique *et al.* (1985). Fruit pulp (10 g) was homogenized in 75 ml distilled water for 5 min in a Waring Blender. Larger particles were removed by filtering the homogenate by suction; the filtrate was made up to 100 ml with distilled water. Samples were filtered through Sep-Pak C18 (Waters Associates) and 5 ml of filtrate were made up to 25 ml with distilled water. This solution was filtered again through a 0·45 μm Millipore filter and 25 μl injected into the chromatograph.

The chromatography was performed with a Waters liquid chromatograph equipped with a Model R 401 differential RI detector. Sample injection was made with a Model U6 K injector and a Model 6000 A pump was used. The column utilized was a Sugar-Pak (Waters) working at 90°C. A Hewlett-Packard 3390 A integrator was used to record and quantitate the peaks for comparison by the external standard method.

Standard mixtures of carbohydrates, obtained from commercial sources (Merck and Sigma), were prepared in deionized water. Concentrations of the standard carbohydrates were similar to the real amounts of these products in apples.

The mobile phase was bidistilled deionized water filtered through a Millipore 0·45 μm filter and degassed prior to the analysis. Flow rate was 0·8 ml min^{-1}. Fruits were analyzed in duplicate each month during their storage time. Twenty-four apples were removed from each plot and two times twelve fruits were sliced and homogenized. Portions of these mixtures were separately used for the chromatographic analysis. Duncan's Multiple Range test was used to determine differences between treatments using each carbohydrate.

RESULTS AND DISCUSSION

The initial composition of both apple cultivars is shown in Table 2. High performance liquid chromatography gave rapid, accurate and reproducible results for the determination of individual carbohydrates. A typical chromatographic separation is shown in Fig. 1. The resolution values between successive carbohydrate peaks were as follows: $R_s = 3{\cdot}12$, sucrose–glucose; $R_s = 2{\cdot}33$, glucose–fructose; $R_s = 4{\cdot}23$, fructose–sorbitol.

The changes in carbohydrate content during storage of both apple cultivars are shown in Tables 3 to 6. Fructose was the major reducing sugar and sorbitol levels were lower than those of glucose and fructose during all

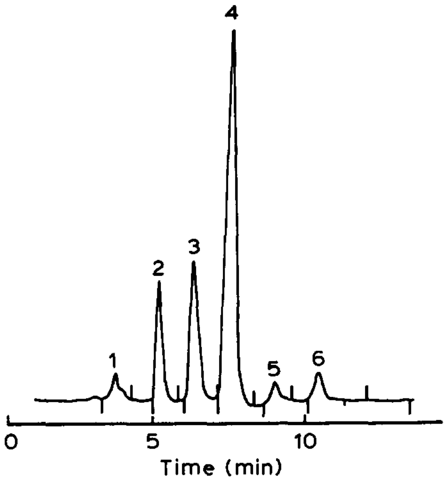

Fig. 1. HPLC chromatogram of carbohydrate compounds in apples. 1, solvent. 2, sucrose. 3, glucose, 4, fructose. 5, unknown. 6, sorbitol.

storage times for treated and untreated apples. Sucrose, fructose and glucose increased during ripening, sucrose 2·6-fold (untreated fruits).

Chemical treatment had a highly significant effect on sucrose content. At the second month of storage, the amounts of sucrose in treated fruits were lower than the corresponding values of untreated fruits; only 'Golden Delicious' apples treated with thiophanate-methyl showed an important increase, threefold the initial sucrose value. There were no significant

TABLE 2
Characteristics[a] of Apples Before the Fungicide Treatments

Characteristic	*cv. 'Golden Delicious'*	*cv. 'Starking'*
Size (mm)	79·5 ± 0·5	72·1 ± 0·4
Weight (g)	222·2 ± 0·3	170·6 ± 0·3
Pulp rupture force (kg)	5·9 ± 0·7	4·0 ± 0·6
Soluble solids (%)	12·9 ± 0·4	12·8 ± 0·7
Reducing sugars (%)	13·0 ± 1·1	11·0 ± 0·9
Total sugars (%)	14·5 ± 0·6	12·5 ± 0·8
Sucrose (%)	1·4 ± 0·4	1·4 ± 0·9
pH	3·8 ± 0·2	4·1 ± 0·1
Titratable acidity (mEq per 100 g)	3·9 ± 3·0	4·2 ± 2·1
Total phenols (%)	2·7 ± 2·5	2·3 ± 1·9
O-diphenol oxidase activity (μ mol per min per g)	7·2 ± 2·4	2·5 ± 2·5

[a] Values are the means of ten fruits ± standard deviation.

TABLE 3

Effect of Post-havest Fungicide Treatment on Sucrose Content (g per 100 g of Fruit) of Stored Apples

Cultivar	*Treatment*	*Months of storage*						
		0	*1*	*2*	*3*	*4*	*5*	*6*
'Golden Delicious'	Untreated fruits	1·47	2·73	3·74	2·36	1·45	1·20	1·14
	Benomyl	—	2·71	1·99*	1·29*	2·01*	1·00	0·34**
	Carbendazim	—	2·67	1·84*	1·60*	1·07	1·69	1·59
	Imazalil	—	2·81	0·86**	1·47**	1·26	1·54	0·88
	Thiophanate-methyl	—	2·53	4·53*	1·25**	1·93*	1·80*	0·45**
	Thiabendazole	—	4·29*	2·14*	1·72*	0·96**	1·26	1·52
'Starking'	Untreated fruits	1·45	2·08	3·19	2·11	1·58	0·97	1·25
	Benomyl	—	2·55	1·35**	1·09*	0·63*	0·44	0·39*
	Carbendazim	—	1·43*	0·88**	1·17*	0·60*	0·79	0·74
	Imazalil	—	2·54	1·29**	2·01	1·13	0·73	0·65
	Thiophanate-methyl	—	2·13	1·35**	0·85*	0·74*	0·95	0·45*
	Thiabendazole	—	2·89*	1·25**	1·37*	0·57*	0·84	1·07

*, **, Significantly different from control at 5% or 1% levels, respectively.

TABLE 4
Effect of Post-harvest Fungicide Treatment on Fructose Content (g per 100 g of Fruit) of Stored Apples

Cultivar	*Treatment*	*Months of storage*						
		0	*1*	*2*	*3*	*4*	*5*	*6*
'Golden Delicious'	Untreated fruits	8·95	11·7	10·6	8·63	7·68	6·53	6·30
	Benomyl	—	10·7*	8·20**	7·23*	9·62*	4·99**	3·23**
	Carbendazim	—	8·35**	9·22*	9·50*	4·26**	8·74*	6·42
	Imazalil	—	12·9*	10·2	6·28**	6·65*	6·90	4·61*
	Thiophanate-methyl	—	9·03**	11·7*	10·0*	8·90*	7·20	3·05**
	Thiabendazole	—	15·6**	8·73*	9·35	4·23**	5·81	6·84
'Starking'	Untreated fruits	6·89	11·4	9·91	7·22	8·39	5·79	6·25
	Benomyl	—	11·5	7·92*	5·07*	6·78*	4·58*	3·29**
	Carbendazim	—	10·1*	6·48**	6·17*	7·11	5·85	5·76
	Imazalil	—	13·5**	17·4**	8·04*	6·99*	5·61	3·65**
	Thiophanate-methyl	—	11·2	15·8**	5·47*	6·33**	6·36	3·46**
	Thiabendazole	—	12·5*	7·14*	9·40**	7·78*	5·42	5·95

*, **, Significantly different from control at 5% or 1% levels, respectively.

TABLE 5

Effect of Post-harvest Fungicide Treatment on Glucose Content (g per 100 g Fruit) of Stored Apples

Cultivar	*Treatment*	*Months of storage*						
		0	*1*	*2*	*3*	*4*	*5*	*6*
'Golden Delicious'	Untreated fruits	3·28	3·62	2·54	2·96	2·57	1·87	2·25
	Benomyl	—	3·33	2·20	2·55	2·18	1·77	0·88**
	Carbendazim	—	2·58*	2·41	2·10*	1·48*	2·33*	1·75*
	Imazalil	—	3·92	1·20**	1·79*	1·80*	2·12	1·39*
	Thiophanate-methyl	—	2·82	2·91	3·22	2·16	2·35*	0·88**
	Thiabendazole	—	4·73*	2·34	2·68	1·27*	1·65	1·77*
'Starking'	Untreated fruits	3·21	3·76	2·23	3·59	2·85	2·43	3·00
	Benomyl	—	5·13**	3·13*	2·10*	2·55	2·50	1·32**
	Carbendazim	—	4·52*	2·70	2·92	2·91	3·01	2·04*
	Imazalil	—	2·83*	3·05*	2·26*	2·78	2·60	2·04*
	Thiophanate-methyl	—	4·30	2·37	2·42*	2·46	3·24*	1·79**
	Thiabendazole	—	4·60*	2·75	4·03	1·29*	2·51	2·59

*, **, Significantly different from control at 5% or 1% levels, respectively.

TABLE 6
Effect of Post-harvest Fungicide Treatment on Sorbitol Content (g per 100 g of Fruit) of Stored Fruit

Cultivar	*Treatment*	*Months of storage*						
		0	*1*	*2*	*3*	*4*	*5*	*6*
'Golden Delicious'	Untreated fruits	0·84	0·55	0·41	0·43	0·34	0·58	0·48
	Benomyl	—	0·56	0·34*	0·24*	0·72**	0·48	0·18**
	Carbendazim	—	0·71*	0·43	0·31	0·72**	0·65	0·46
	Imazalil	—	0·40	0·26**	0·37	0·71**	0·48	0·28*
	Thiophanate-methyl	—	0·46	0·51**	0·49	0·53*	0·63	0·27*
	Thiabendazole	—	1·62**	0·45	0·32	0·27	0·39*	0·55
'Starking'	Untreated fruits	0·96	0·43	0·33	0·26	0·37	0·32	0·59
	Benomyl	—	0·96**	0·30	0·23	0·31	0·22	0·12**
	Carbendazim	—	0·46	0·19*	0·23	0·26	0·37	0·32*
	Imazalil	—	0·40	0·37	0·32	0·40	0·43	0·42*
	Thiophanate-methyl	—	0·32*	0·38	0·19*	0·35	0·53*	0·23**
	Thiabendazole	—	0·93**	0·29	0·42*	0·34	0·28	0·41*

*, **, Significantly different from control at 5% or 1% levels, respectively.

differences between cultivars in the evolution of sucrose content at the post-climacteric stage.

Fructose contributed about 80% to the total reducing sugar content of ripe untreated fruit and fructose data were significantly different from treated apples. The effect of each chemical treatment was slightly different for the total reducing sugars of both apple cultivars; imazalil and thiophenate-methyl caused greater amounts at the same time (1–2 months) of storage, while the other treatments (benomil, carbendazim and thiabendazole) showed an opposite effect. At the end of storage, all treated fruits were excessively ripe. There were no significant differences in glucose content between untreated (control) and treated ripe fruits (Table 5); however, chemical treatments had a highly significant effect on fructose levels (Table 4).

The evolution of sorbitol is shown in Table 6. Apples showed a great fall in sorbitol content during ripening and, at the same time, an increase in fructose was observed because there was a sorbitol to fructose transformation (Whiting, 1970). This fall in sorbitol level was increased by fungicide treatments. Apples treated with carbendazim, imazalil and thiophanate-methyl showed lower levels of this carbohydrate than untreated fruits.

Similar effects of pesticides were previously reported by Rouchoud *et al.* (1984), who observed an increase in the total free sugar content in apples treated with combined fungicides and insecticides during the growing-season.

This work indicates that post-harvest treatments with fungicides have a positive effect on the biochemical composition of sugars in both studied apple cultivars. The increase of total sugar content produces an excessive ripening, diminishing the storage time of fruits. This effect was greater in apples treated with imazalil, which showed excessive wrinkling and peel dehydration at an early storage time. Fruits treated with benzimidazole fungicides (benomyl, carbendazim, thiophanate-methyl and thiabendazole) maintained good quality in the first stages of storage although they showed fast ripening caused by the post-harvest treatments.

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