

AN UNKNOWN ORGANIC ACID
THAT ACCUMULATES DURING CA STORAGE

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Introduction

Controlled Atmosphere (CA) storage being based on the lowering of the respiratory activity, it is normal that it has important effects on the metabolism of the stored produces (1, 4, 5). A rather general survey of these changes was performed in our laboratory, with the attention focussed especially on organic acids.

Material and Methods

Material: The main experiment the results of which are reported in this paper was done on McIntosh apples, but a comparative study was also made with Golden Delicious apples. In order to work on healthy and physiologically defined fruits, these were picked by ourselves, manually, at the date determined by physiological observations, and handled very carefully between the orchard and the laboratory.

The fruits were then put in polyethylene bags (made of 5 micron-thick film) containing each a sample of 20 fruits, which had been weighed. After the bags were air-tight sealed, they were placed in a room cooled at 4°C. This is the laboratory-scale of Marcellin's "physiological packaging" (2). The composition of the atmosphere inside the bags was found to maintain itself at the O₂/CO₂ levels of 3/5.

Extraction and analysis of soluble compounds

Of each of the 20 apples of a sample taken out of the storage room, four longitudinal sectors (approximately 5 mm thick on the circumference) are taken at the four cardinal points, weighed, deprived of the peel and internal cartilaginous walls of the cavity, and ground in ethanol (4:1, v:w). The filtered extract was then brought to a known volume with 80% ethanol. Aliquots of this extract were used for the analyses.

(In this study, carbohydrates, phenols and proteins were determined, and some enzymes assayed. However, only results concerning organic acids are reported here.)

For the analysis of organic acids, the ethanol extract was evaporated to dryness, then the residue was redissolved in 100% anhydrous methanol; aliquots were then esterified with methanol-BF₃ for 16 hours at room temperature. The methylated acids were recovered, by partition, in chloroform, and analyzed by gas chromatography (3). The "apparent percentage" of each peak, i.e. the ratio of its surface area to the sum

of the areas of all peaks, was calculated (no correction for the response of the detector to each compound). In the search for the identity of the unknown compound, nuclear magnetic resonance was used.

Results

Gas chromatographic analysis showed the existence of an unknown acid, which came out soon after malic acid. Its peak was well defined, and corresponded to no known standards injected. NMR analysis revealed the presence of 4-carbon compound with two carboxyl groups, and there was a signal which could correspond to an amine group, however not confirmed.

When apparent percentages were calculated, it was interesting to see that there was a decrease in malic acid from day 0 to day 139 (table 1), and the occurrence of a corresponding amount of the unknown acid. This was confirmed in other experiments with McIntosh and Golden Delicious apples.

The only remarkable exception was seen when rapid analyses were made as soon as CA stored apples were taken out of the storage room: the first change occurred as a balance between malic and succinic acid (table 2), then the percentage of the latter acid decreased and that of the unknown acid increased.

Discussion

The main point that emerges from the data reported here is that, contrarily to the general opinion, succinic acid does not seem to markedly accumulate during the time when the fruits are in the controlled atmosphere at low temperature. Succinic acid only appears when the fruits are brought back to air. And its accumulation seems to be rather fugitive, while another, yet unidentified, C-4 organic acid - which may be bearing an amino group - is forming at the expenses of malic acid. We are still trying to identify the unknown acid, and to understand this process of interconversion between it and malic acid.

Literature cited

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Table 1. Apparent percentages of the three main organic acids over the whole period of CA storage.

<u>Days in CA</u>	<u>Apparent percentage</u>		
	<u>Succinic</u>	<u>Unknown</u>	<u>Malic</u>
0	-	97	3
139	2	80	18

Table 2. Apparent percentages (at 4 and 20° C) of the three main organic acids after the return to air after 139 days in CA.

<u>in CA</u>	<u>Day</u>		<u>Apparent percentage</u>					
	<u>in air</u>		<u>Succinic</u>		<u>Unknown</u>		<u>Malic</u>	
			4°	20°	4°	20°	4°	20°
139	0		2		19		80	
	1		10	24	61	55	23	18
	3		14	7	63	50	22	42
	6		18	10	59	43	20	51