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CHANGES IN THE ORGANIC ACIDS OF JONATHAN APPLES DURING COOL STORAGE IN RELATION TO THE DEVELOPMENT OF BREAKDOWN

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Abstract—Changes in oxalacetic, α-ketoglutaric and pyruvic acids, non-volatile organic acids, and titratable acidity of Jonathan apples during storage at −1° were examined. A storage treatment which increased the rate of water loss and significantly delayed the development of breakdown did not affect the amounts or changes with time of any of the acids. The results indicate that, in contrast with published data, in our results there is no direct causal relationship between oxalacetic acid and breakdown.

INTRODUCTION

SERIOUS losses may occur during cool storage of some varieties of apples due to the development of the physiological disorder, internal breakdown. One form of this disorder, low-temperature breadown, develops in susceptible varieties after storage for relatively short periods below 3° but above the freezing point of the fruit.[1] Low-temperature breakdown appears as brown flesh in the cortex, but when severe it may affect the whole fruit.

New information on the physiology of this disorder has recently been reported.[2,3] Scott *et al.*[2] found that storage of Jonathan apples at 0° over anhydrous calcium chloride, a treatment which promotes a higher rate of water loss, retarded the development of breakdown. Hulme *et al.*[3] showed that, in Cox's Orange Pippin apples, oxalacetate content increased during storage at 0° until the onset of the disorder. They also showed that warming the fruit for 5 days at 15°, after storage for 6 or 8 weeks at 0°, resulted in a rapid decrease of oxalacetate and a considerable delay in the onset of breakdown during subsequent storage at 0°. The oxalacetate content rose slowly during this latter period of storage. They suggested that low-temperature breakdown in apples is caused by an interference in the operation of the Krebs cycle.

In the work reported in this paper we have examined the changes during storage at −1° in α-keto acids, non-volatile organic acids, and titratable acidity in fruit having different rates of weight loss and, consequently, different rates of development of breakdown. The aim was to find if the level of breakdown was related to any of these acids and in particular to test the hypothesis that a high incidence of low-temperature breakdown results from a high content of oxalacetate.

[1] W. M. CARNE, *Australia, Commonwealth Sci. Ind. Res. Organ.* Bull. No. 238, p. 52 (1948).
[2] K. J. SCOTT, E. G. HALL, E. A. ROBERTS and R. B. WILLS, *Australian J. Exp. Agr. Animal Husbandry* 4, 253 (1964).
[3] A. C. HULME, W. H. SMITH and L. S. C. WOOLTORTON, *J. Sci. Food Agr.* 15, 303 (1964).

RESULTS

Time of Picking in Relation to Respiratory Activity

Trends in respiratory activity of developing fruit on the tree were followed by measuring the respiration rates of single apples 24 hr and 48 hr after picking. It was intended to use these trends to indicate the development of an "on-tree" climacteric[4, 5] so that fruit required for the analytical and storage studies could be picked at three dates, corresponding to pre-climacteric, climacteric and post-climacteric stages of respiratory activity.

The data presented in Fig. 1 shows that the respiration rate decreased as the fruit developed until 1 March, when the first picking was made. The time taken for the fruit to reach a post-harvest climacteric also decreased to this date (Table 1). The measurements made after this

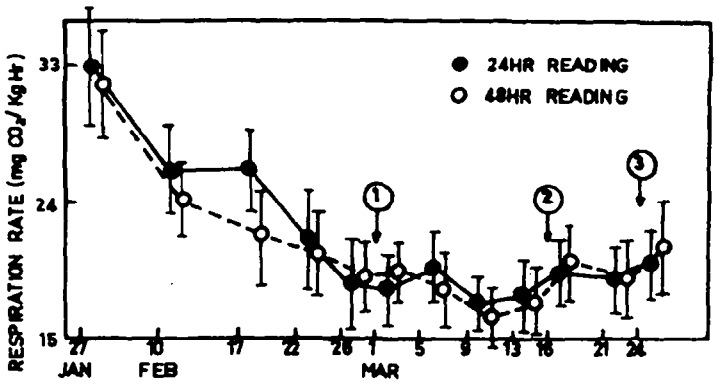

FIG. 1. TRENDS IN THE MEAN RESPIRATION RATES OF APPLE SAMPLES DURING MATURATION. Measurements were made at 20°, 24 and 48 hr after picking. The bars through each point represent estimates of the standard deviation of the population ($n=24$) and the ringed numbers indicate the dates on which the storage samples were picked.

date showed no clear trend and the "on-tree" climacteric was not apparent. The second and third pickings were made at the dates shown in Fig. 1 but due to the failure to measure the "on-tree" climacteric they were made on the basis of ground colour of the skin. Based on this criterion, the second picking was considered optimum for cool storage.

TABLE 1. NUMBER OF DAYS AT 20° FOR THE FRUIT TO REACH A RESPIRATORY CLIMACTERIC

Date of picking	27 Jan.	10 Feb.	17 Feb.	1 Mar.	5 Mar.
No. of days ($n=12$)	73·1	33·9	18·5	12·3	11·1
S.D.*	±21·2	±21·2	±13·7	±4·09	±5·44
Date of picking	**9 Mar.**	**16 Mar.**	**21 Mar.**	**24 Mar.**	
No. of days ($n=12$)	7·4	8·2	7·5	7·7	
S.D.*	±2·32	±2·16	±1·84	±2·47	

* Standard deviation.

[4] A. C. HULME, J. D. JONES and L. S. C. WOOLTORTON, *Proc. Roy. Soc. London* **158B**, 514 (1963).
[5] E. C. MAXIE, P. B. CATLIN and H. T. HARTMAN, *Proc. Am. Soc. Hort. Sci.* **75**, 275 (1960).

Incidence of Breakdown

The fruit was stored under conditions which provided two widely different rates of weight loss (3·3:1). The rates were linear throughout the storage period and were the same for each picking (Fig. 2).

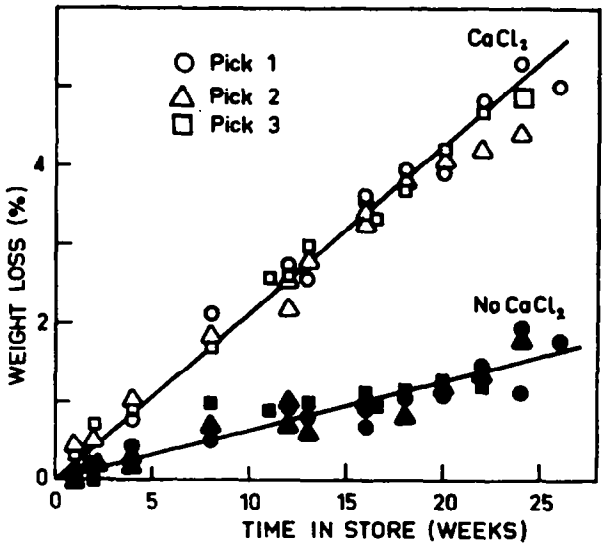

FIG. 2. WEIGHT LOSS DURING STORAGE.

Table 2 shows that the development of breakdown was delayed by the high weight loss treatment. An analysis of variance was carried out on the data for each picking and the difference in the levels of breakdown between the two treatments was found to be highly significant ($p < 0{\cdot}001$) for all pickings. The effect of time in store on the levels was also found to be highly significant ($p < 0{\cdot}001$) in all pickings.

TABLE 2. NUMBERS OF FRUIT AFFECTED WITH BREAKDOWN

Pick	Weight loss treatment	Storage time at −1° (weeks)								
		11	12	13	16	18	20	22	24	26
1	Low			14	12	8	17	19	28	28
	High			5	2	4	8	11	8	20
2	Low		7	11	20	19	26	29	32	
	High		11	7	5	7	4	20	14	
3	Low	17	16	23	29	28	29	35		
	High	4	10	13	14	12	14	20		

The values given are the total number of fruit in two units of twenty-four fruit affected with breakdown after a post-storage holding period of 1 week at 20°.

The data were fitted by multiple linear regressions to relate breakdown to storage period, weight loss and original weight at picking. There were six classes (3 pickings × 2 treatments) and within class regressions were fitted. There were no significant differences between the

corresponding regression coefficients for the six classes. For each picking the differences between the treatments were accounted for by the differences in weight loss. Variations in original weight did not fully account for the differences in breakdown between the pickings.

α-Keto Acids

The α-keto acids found were oxalacetic, α-ketoglutaric and pyruvic acid, with glyoxylic acid present in trace amounts. Estimation of these acids was carried out at intervals during storage until visible signs of breakdown were found in the fruit immediately on removal from cool storage, which occurred after 16 weeks at low temperature.

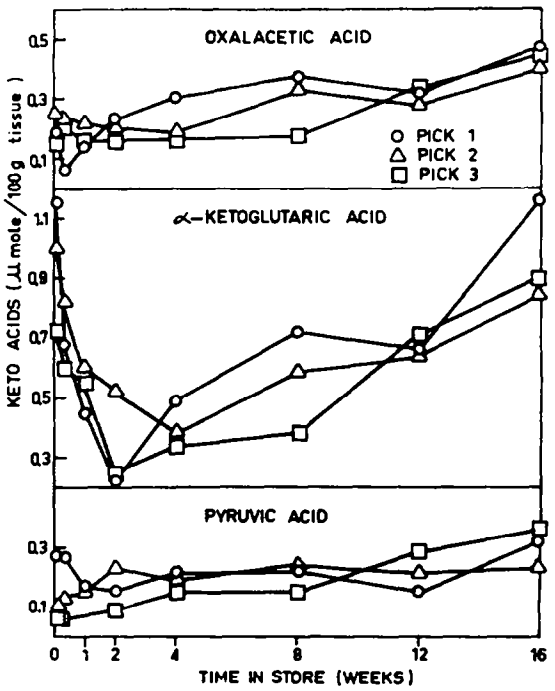

FIG. 3. CHANGES IN α-KETO ACIDS DATA DURING COOL STORAGE. THE DATA PLOTTED ARE THE MEANS OF THE HIGH AND LOW WEIGHT LOSS TREATMENTS.

An analysis of variance was carried out on the combined data from the three pickings. This showed that there was no significant difference in the content of any acid due to the storage treatment, but that there was a significant change ($p < 0{\cdot}001$) in the amounts of each acid with time in store (Fig. 3). Oxalacetic acid increased during storage for all pickings, but in pickings 2 and 3 there was a lag of 4 to 8 weeks before the level of acid began to rise. The level of α-ketoglutaric acid fell rapidly in all pickings and then rose to approximately the original level after 16 weeks in store. Pyruvic acid increased gradually during storage. Figure 3 also shows that the amounts of α-ketoglutaric and oxalacetic acids tended to be less in the later pickings, especially from 4 to 8 weeks in store: but as there was no replication of pickings the significance of these differences could not be evaluated.

Titratable Acidity

Titratable acidity declined gradually during storage in all pickings (Fig. 4), and there were no differences due to the storage treatment.

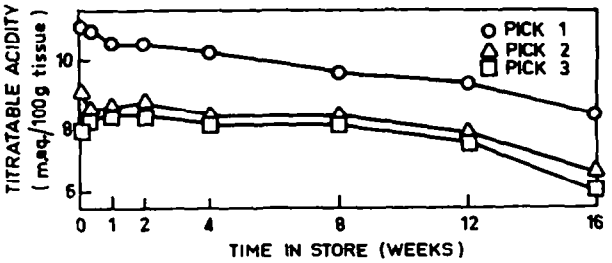

FIG. 4. CHANGES IN FREE ACIDS DURING STORAGE. THE DATA PLOTTED ARE THE MEANS OF THE HIGH AND LOW WEIGHT LOSS TREATMENTS.

Non-volatile Organic Acids

The presence of malic, shikimic, quinic, citric, succinic, citramalic, aspartic and glutamic acids in alcohol extracts of the apple tissue was established, but only the first three acids could

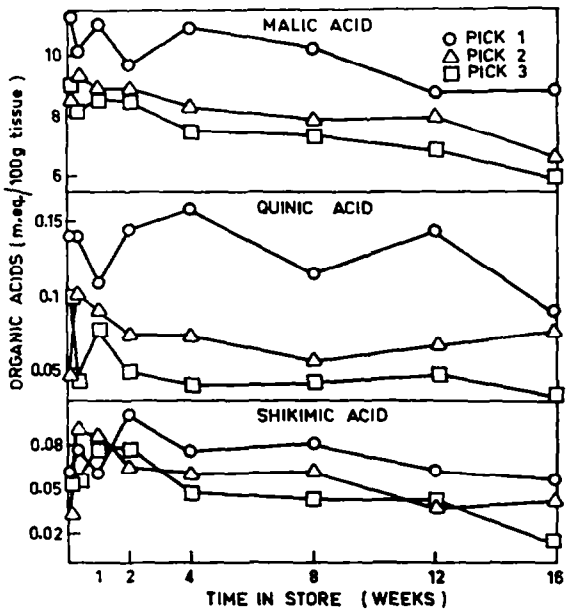

FIG. 5. CHANGES IN NON-VOLATILE ORGANIC ACIDS DURING STORAGE. THE DATA PLOTTED ARE THE MEANS OF THE HIGH AND LOW WEIGHT LOSS TREATMENTS.

be quantitatively assayed (Fig. 5). Storage treatment had no consistent effects on changes in the contents of these acids but significant changes with time in storage were found. The loss of malic acid was approximately equal to the decrease in titratable acidity.

DISCUSSION

The data presented in Fig. 2 and Table 2 show that

(1) the rate of weight loss and the rate of development of breakdown were the same in fruit of all pickings,
(2) that the only difference between pickings was the earlier onset of breakdown in the more mature fruit, and
(3) that the higher weight loss treatment delayed the onset of the disorder without changing its rate of development.

These observations suggest that the nature of the breakdown was the same in all pickings and was not changed by the treatment with higher weight loss.

Changes in the levels of the three α-keto acids in Jonathan apples during storage (Fig. 3) show a pattern similar to that reported by Hulme *et al.*[3] for Cox's Orange Pippin but the higher weight loss had no significant effect on the amounts, or time courses of changes in the α-keto acids. This indicates that there is not a direct causal relationship between the content of the α-keto acids, particularly oxalacetic acid, and the onset of breakdown.

Wilkinson[6] found that storage in a dry atmosphere decreased the permeability of apples to gas exchange and, conversely, storing the fruit in a wet atmosphere increased the permeability. Increased evaporation of water could act by carrying from the fruit a volatile breakdown initiator. On the other hand a decrease in permeability could retard the escape of a volatile inhibitor. We have some evidence (unpublished) which suggests that there are differences in the production of volatiles by apples stored in atmospheres of low and high humidity.

Further investigation of the possible relationships between organic acid metabolism, apple fruit volatiles, rate of evaporation and breakdown are being investigated with emphasis on changes which occur before the onset of the disorder.

EXPERIMENTAL

Measurement of Respiration Rates

The respiration rates of single fruit at 20° were measured by the colorimetric method of Claypool and Keefer.[7] CO_2 concentrations in the respiration containers were maintained at less than 0·3% for preclimacteric fruit by ventilation with humidified air.

Storage Unit

The unit used for assessing the development of breakdown and for the analysis of the organic acids consisted of a composite sample of twenty-four fruit obtained from twelve trees from Bilpin, N.S.W. Fruits of comparable size and maturity were selected at each picking. To prevent pre-harvest fruit drop a spray of 20 ppm 2,4,5-trichlorophenoxypropionic acid was applied at a rate of 3 gal per tree on 8 March, 1965.

Analytical Methods

Each apple in a unit was cored and cut longitudinally into twelve sectors. Single sectors from each apple were combined to form a composite sample which was frozen in liquid air and then ground in an electric grinder at −20°. Aliquots of the frozen powder were taken for the various determinations.

The titratable acidity was measured by titrating 25 g of tissue mixed with water (100 ml) to pH 8·2 with 0·1 N NaOH.

The α-keto acids were determined by the method of Isherwood and Niavis[8] as adapted for apple tissue by Hulme *et al.*[3] This involved extracting samples (50 g) with 0·6 M HPO_3 at 0° and separating the acids as the 2,4-dinitrophenylhydrazones by paper chromatography. The individual acids were measured colorimetrically using a Bausch and Lomb Spectronic 20.

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[7] L. L. CLAYPOOL and R. M. KEEFER, *Proc. Am. Soc. Hort. Sci.* **40**, 177 (1942).
[8] F. A. ISHERWOOD and C. A. NIAVIS, *Biochem. J.* **64**, 549 (1956).

Samples (100 g) for the determination of the non-volatile organic acids were freeze-dried at a sublimation temperature of −30° to a final product temperature of 50° and stored in sealed containers at 0° until analysed. The freeze-dried samples were extracted three times with 80% ethanol (100 ml) in a food blender at room temperature. The filtered extracts (541 Whatman paper) were combined and one-half of the sample was loaded onto a 22 × 0·7 cm column of Permutit De-acidite FF (< 200 mesh) anion exchange resin in the acetate form. The other half of the sample was held in reserve at −15°. The acids were eluted with a linear gradient of acetic and formic acids (Hulme and Wooltorton[9]) generated by a four-chambered apparatus using variable gradient of the type used by Peterson and Sober.[10] The four chambers initially contained 125 ml of deionized water, 2·5 N acetic acid, 2·5 N acetic acid and 6 N formic acid respectively. The flow rate was 1–1·5 ml/min. Fractions (5 ml) were collected and dried by Palmer's[11] method. The fractions containing titratable amounts of acid were detected by adding 1 ml of 0·001% phenolphthalein in 0·002 N NaOH to each collection tube (de Moura and Dostal[12]). These fractions were titrated to pH 8·2 with 0·02 N NaOH.

The analytical data have been reported on the basis of the fresh weight of the fruit at picking.

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[12] J. DE MOURA and H. C. DOSTAL, *J. Agr. Food Chem.* **13**, 433 (1965).