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Peroxidase Activity in Golden Delicious Apples as a Possible Parameter of Ripening and Senescence

Total peroxidase activity was estimated in Golden Delicious apples during storage at 3–4°C, under a controlled atmosphere. As a function of storage time, peroxidase gave two peaks, the first corresponding to climacterium and the second to the start of senescence.

Ribonuclease, protease, and peroxidase have often been associated with senescence (Sacher, 1973). However, Sacher and other authors do not clearly differentiate between ripening and senescence (e.g., Hulme, 1970, 1971). Rate of respiration is a parameter of aging for various kinds of apples stored at 12°C or above. At 3–4°C, the rate of respiration is practically constant (Fidler, 1973). For Golden Delicious apples, Struklec (1970) came to the same conclusion. Biale (1964) and Fidler (1973) have attempted to distinguish ripening and senescence, but only schematically. We investigated whether peroxidase could be used as a parameter to distinguish ripening and senescence in Golden Delicious apples stored at 3–4°C in a controlled atmosphere.

MATERIALS AND METHODS

Golden Delicious apples (about 280 kg), harvest 1973, of uniform color and size (about 70–80 mm diameter) were purchased from a fruit grower at Puiflyk, Netherlands, and stored at 3–4°C under controlled atmosphere (3–4% O_2:7–8% CO_2) (Gorin, 1973; Gorin et al., 1975). Apples were sampled at various intervals (storage samples, Table I) (Gorin et al., 1975).

Acetone (Clements') powders were prepared as before (Gorin, 1973), except that they were kept at −40°C and under nitrogen to avoid loss of peroxidase activity.

Peroxidase was estimated during September–October 1974. Thus, the powder from the first sampling of fruits was stored for about a year and the last about 4 months.

The moisture content of the acetone powders (Table I) was estimated as before (Gorin, 1973).

The nitrogen content of each powder (Table I) was estimated by the Element-Analytical Department of the Institute for Organic Chemistry TNO (Utrecht) using a Heraus Rapid N combustion apparatus. This is a modified Dumas method developed by Merz (1968) and the maximum deviation is 0.2% (w/w).

The nitrogen content of the powder was not corrected for differences in moisture, since their effect on the results was negligible.

Table I. Nitrogen (% w/w) and Moisture (% w/w) Content of Acetone Powders from Fruits Stored for Different Periods[a]

Storage time, days	Moisture[b] $\bar{x}$	Moisture[b] s (±)	Nitrogen
0	0.78*		1.09
26	0.95	0.06	1.00
54	0.18	0.05	1.02
96	0.46	0.12	0.99
124	1.47	0.13	1.01
152	0.77	0.02	1.09
180	0.43	0.08	0.78
208	0.48	0.04	1.01
236	0.61	0.06	0.77

[a] Fruit was put into storage on Oct. 3, 1973. [b] $\bar{x}$ = mean; $s = (\Sigma d^2/n - 1)^{1/2}$, n = number of estimates, which was 3 except for the asterisked entry where it was 2.

The enzyme solution was prepared by stirring in an ice bath a suspension of powder (1.5 g) with 6.7 mmol/l. phosphate buffer (pH 7) (15 ml). After 120 min, it was centrifuged at 2°C for 1 hr at 49000*g*. The supernatant constituted enzyme solution 1.

The residue was resuspended, stirred, and centrifuged as before. The supernatant was enzyme solution 2. The process was repeated to obtain enzyme solution 3. Solution 4 contained insignificant amounts of protein, and was therefore ignored.

The protein content of each solution was estimated by a modified method of Lowry et al. (1951) as described by Bailey (1967).

Peroxidase activity was estimated as described by Lück (1965), slightly modified. The amounts of reagents were decided after preliminary trials.

The sample cuvette contained enzyme solution (0.2 ml), the phosphate buffer (pH 7) (1.8 ml), an aqueous solution

Table II. Peroxidase Activity of Solutions 1, 2, and 3 from Acetone Powder of Fruits Extracted when Put into Storage (Day 0)

Solution	Protein concn of enzyme soln, mg/ml[a]	Peroxidase act., arbitrary units $\bar{x}$	s (±)	CV (±), %
1	0.85	0.328	0.061	19
2	0.75	0.167	0.024	15
3	0.52	0.090	0.015	17
Total		0.585	0.067	11

[a] Average of two estimates, which were similar in value; $\bar{x}$, average of triplicate assays; s as in Table I; CV = $s100/\bar{x}$. In the total row, standard deviation of the sum = $(s_1^2 + s_2^2 + s_3^2)^{1/2}$ (Spiegel, 1972), where s_1, s_2, and s_3 are s values of first, second, and third enzyme solutions, respectively. Peroxidase was measured in terms of an increase in absorbance (see Materials and Methods).

(1% w/v) of *p*-phenylenediamine (0.1 ml), and H_2O_2 solution (3 mmol/l.; 0.3 ml). The reference cuvette contained the same reagents as the sample, but distilled water instead of H_2O_2.

The absorbance (A) at 485 nm (1-cm path length; 25°C) was recorded in a double-beam spectrometer (Beckmann, DB-GT). The initial slope of the line (ΔA during the first minute) constituted an arbitrary unit of activity, which was corrected to 1 mg of protein in the enzyme solution.

RESULTS

As an example of the type of data, Table II records the peroxidase activity of the three enzyme solutions from the powder of fruits when put in storage (day 0).

In powders from later samples of apples, the proportion of total protein always fell from solution 1 to solution 3, but the proportion of total peroxidase activity was often less in solution 2 than in solution 3, though always the most in solution 1. (A complete Table II is provided as Table 2a in the Supplementary Material.)

Figure 1 records peroxidase activity during storage of fruits harvested in 1973, as well as some data from 1972. The second peak of total peroxidase activity is practically determined by one point (208 days) which could put in doubt the second stage. However, more recently we have found a similar increase in protease at 152 days, reaching a peak at 180–208 days (unpublished data).

DISCUSSION

Table II shows the importance of not confining enzyme assay to solution 1. Not all the protein was extracted from the powder in the first solution.

There were two peaks of total peroxidase activity during storage. The first peak corresponds to ripening and the second to senescence. The first could be associated with breakdown of hormones that retard ripening (Frenkel, 1974) and the second could be a defense mechanism against hydroperoxides. The peak at ripening was reproducible (harvests of 1972 and 1973) even though the apples were not picked on the same date nor at the same biological age. Storage of apples from the 1972 harvest ended before the second peak could fall off. Senescence can be detected after both the 1972 and 1973 harvests had been stored for 180 days (Figure 1), if the start of the senescence is identified with the start of the second peak. It would be interesting to know whether the sequence of peroxidase activities is genetically controlled.

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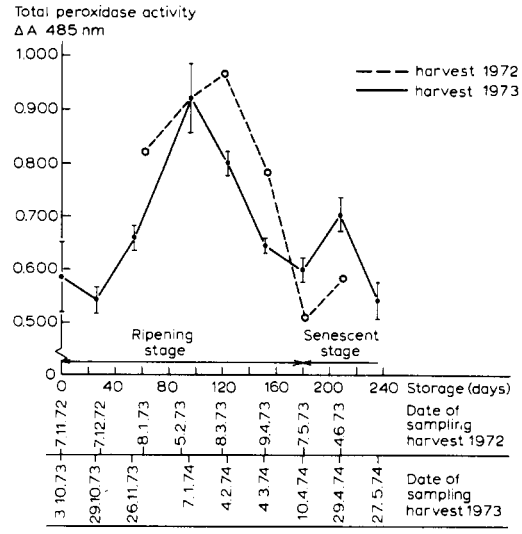

Figure 1. Total peroxidase activity during storage of Golden Delicious apples. Vertical bars represent s (total) (see Table II). Peroxidase activity is expressed in arbitrary units (see Materials and Methods).

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Supplementary Material Available: Table 2a giving complete data on peroxidase activity of the three enzyme solutions, two pages. Ordering information is given on any current masthead page.

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