Phytochemistry, 1976, Vol. 15, pp. 625–626 Pergamon Press. Printed in England.

CHANGES IN THE GLUCANS OF RIPENING APPLES

IAN M. BARTLEY
East Malling Research Station, East Malling, Maidstone, Kent, ME19 6BJ, England

(*Revised received* 2 *January* 1976)

Key Word Index—*Malus domestica*; Rosaceae; apple fruit; glucans; ripening; cell wall composition.

Abstract—The noncellulosic glucose content, like the xylose content, of cell walls of cortex tissue of apples showed little change as the fruit ripened and the cellulosic glucose also remained constant. There was a considerable loss of galactose residues from the walls, however, whilst only a small change in arabinose was observed. The starch was rapidly hydrolysed.

INTRODUCTION

The cortex tissue of apple softens as the fruit ripens. Softening is accompanied by the loss of galactose residues from the cell wall and an increase in the polygalacturonide freely soluble in aqueous extracts [1,2], although the loss of galactose does not lead directly to the solubilisation of the polyuronide. Therefore, hydrolysis of other cell wall components may be involved. It has been reported that glucose residues are lost from the cell walls during ripening [1] suggesting that the glucans of the wall are hydrolysed. The work described in the present paper has sought to confirm this observation by the determination of the noncellulosic glucan and cellulose contents of the cell walls in the ripening fruit.

RESULTS AND DISCUSSION

Extraction of starch from cell wall preparations

Starch was extracted from the cell wall preparations by treatment with chloral hydrate [1] and any remaining polymer solubilised by incubation with α-amylase [3]. Hydrolysis of the chloral hydrate extract with M H_2SO_4 released only glucose. Arabinose, xylose and galactose, components of cell wall polymers, were not detected and it was concluded that starch is the only polymer solubilised in this extraction.

Chloral hydrate effectively extracted the starch from the cell wall preparations for there was little release of reducing sugar in the subsequent incubation with α-amylase. If the cell wall preparations were not extracted with chloral hydrate prior to incubation with α-amylase then the weight of cell walls which resulted after a 72 hr incubation with the enzyme was 52% higher by wt than the cell walls prepared using the standard procedure. This indicated that the enzyme alone was unable to completely solubilise all the starch in the cell wall preparations.

Hydrolysis of cell wall preparations

The cell walls were hydrolysed with M H_2SO_4 to release the component noncellulosic neutral sugars for analysis. The total glucose content of the cell walls was determined following a preliminary hydrolysis with 72% (w/w) H_2SO_4 and a secondary hydrolysis with 0.4 M H_2SO_4 [1,2], this procedure is thought to give complete release of neutral sugar residues of the cell wall [4]. Hydrolysis of cell walls with M H_2SO_4 and 72% H_2SO_4 indicated that M H_2SO_4 released 81% of the arabinose, 69% of the xylose and 89% of the galactose of the wall. Hydrolysis of a standard cellulose preparation (Avicel) with M H_2SO_4 and 72% H_2SO_4 released 4.9 and 80.7 mg of glucose/100 mg of cellulose respectively. Thus the glucose released from the cell wall preparations by M H_2SO_4 was predominantly noncellulosic in nature [5].

Changes in cell wall composition in ripening apples

The noncellulosic glucose content of the cell walls showed little change in the ripening fruit and was *ca* 50% of the content of xylose which also remained constant [1,2] (Table 1). Thus the noncellulosic glucan and xylan components of the wall are not hydrolysed during ripening. The cellulosic glucose content of the walls did not change either, confirming earlier observations [6]. Wall glucan hydrolysing activity appears to be absent in the apple since assay of cortex tissue preparations with carboxymethylcellulose, a polysaccharide which contains the β-1, 4-glucosyl linkages thought to be present in cell wall glucans, failed to detect glycosidase activity. (I. M. Bartley, unpublished data). The earlier observations of hydrolysis of the cell wall glucans [1] may derive from the use of different procedures for the determination of the total glucan, including starch (hydrolysis, glucose oxidase) and starch of the preparations (colorimetric).

There was a substantial loss of galactose residues from the cell walls as the fruit ripened whilst the concentration of readily soluble polygalacturonide remained constant for 79 days (Table 1). The fruit started to soften slowly after 49 days and the increased rate of softening noted in the period 79–119 days was accompanied by a *ca* three fold increase in the soluble polygalacturonide concentration. A small decrease was observed in the arabinose content of the walls during ripening.

The results show that two major compositional changes occur in cell walls of the cortex tissue of ripening apples, namely a loss of galactose residues and solubilisation of the high MW polygalacturonide. The loss of galactose residues from the cell wall does not lead directly to the solubilisation of the polygalacturonide sug-

Table 1. Changes in firmness, cell wall composition and starch concentration in ripening apples

Time in store (days)	Fruit firmness (kg)	Soluble polyuronide (mg/g fr. wt)	Neutral sugar content of cell walls (mg/g fr. wt)					Starch (mg Glc/g fr wt)
			Ara	Xyl	Gal	noncellulosic Glc	cellulosic Glc*	
0	4.8	0.10	1 95	0 67	2.75	0 30	3 82	14 98
7	5 3	0.12	2 21	0.73	2.80	0 29	3.83	11.61
28	4 8	0.14	1.96	0 67	2 24	0 30	3 67	3.77
49	4.9	0.12	1 97	0 72	2 07	0 29	3 86	0 62
79	4 4	0 13	1.85	0.64	1 26	0 30	3.77	0
119	3 4	0.35	1.75	0.62	0 97	0 26	3.72	0

* Total cell wall Glc—noncellulosic Glc

gesting that hydrolysis of other as yet unidentified linkages may be involved. Detailed study of the composition of the cell walls [7,8] suggests that the loss of galactose occurs mainly by hydrolysis of the galactan of the primary cell wall, probably through the activity of the β-galactosidase present in the cells [2], whilst the polygalacturonide is derived from the middle lamella.

Starch was rapidly hydrolysed as the fruit ripened (Table 1). α-Amylase activity is present in the cortex tissue cells of ripening apples (I. M. Bartley, unpublished data) and is thought to be responsible for the degradation of the starch because α-amylase is the only glycosidase which is able to hydrolyse starch granules *in vivo* [9].

EXPERIMENTAL

A bulk sample of fruit was obtained on 16th September, 1974 from trees of Cox's Orange Pippin at East Malling Research Station and sorted into groups of 16 apples. The fruit was placed in pairs in polythene tubes each of which was sealed and connected to a line delivering a gas mixture of 2% O_2 at 2.5 l/hr at 3.5°.

Preparation of cell walls. Cortex tissue homogenates were prepared in 5 mM Pi pH 7.2 as before [2]. The preparations were filtered through sintered glass (the filtrate being retained for determination of polyuronide), suspended in 100 mM Pi, M NaCl, 0.1% (w/v) Triton X-100, pH 7 (2 ml/g tissue) and homogenised for 60 sec at maximum speed with an Ultraturrax homogeniser to ensure complete breakage of cells and the release of starch grains. After filtration and washing with H_2O the residue was re-suspended in H_2O (2 ml/g tissue) and stored at −20° until required. 10 ml of preparation was extracted with 35 g chloral hydrate [1] for 48 hr at 20°. After addition of 25 ml of H_2O, the suspension was filtered and the filtrate retained. Starch was recovered from the chloral hydrate soln by precipitation with 4 vols Me_2CO and collected by centrifugation. The residue was washed with H_2O and suspended to 10 g in 100 mM Pi pH 6.5. 7.5 ml of this preparation were incubated with 0.5 ml of a soln of *Bacillus subtilis* α-amylase (Sigma) for 24 hr at 20° under toluene on a bottle incubator. The α-amylase preparation released 100 mg of maltose/min/mg of enzyme at 20° with a soluble starch substrate. The cell walls were collected by filtration, washed with 5 mM Pi pH 7.2, H_2O and then successively with 80% (v/v) Me_2CO, Me_2CO and Et_2O and dried to constant wt. Knee [7,8] prepared cell walls from the cortex tissue of unripe and ripe Cox apples using the above procedure. He found (personal communication) that polyuronide in the 5 mM Pi extracts increased from 0.1 mg/g fr. wt in unripe fruit to 1 mg/g fr. wt in ripe fruit. The glucose content of the extracts was 60 μg/g fr. wt whilst the values for arabinose, galactose and xylose were 70–140, 110–140 and 10 μg/g fr. wt respectively. Ultraturrax treatment of the 5 mM Pi insoluble material with high ionic strength buffer released 30–80 μg polyuronide/g fr. wt and 30–40 μg glucose/g fr. wt.

Hydrolysis of cell walls and estimation of neutral monosaccharides. 5–10 mg of cell walls were suspended in 1 ml M H_2SO_4 and autoclaved at 121° for 1 hr. Mannitol was added as internal standard prior to neutralisation with $BaCO_3$ and the monosaccharides analysed by GLC as their TMSi ether derivatives [1]. To determine total neutral sugar content, 5–10 mg of cell walls were suspended in 0.05 ml 72% (w/w) H_2SO_4 for 23 hr at 20°, diluted to 0.4 M H_2SO_4 and autoclaved at 121° for 1 hr. Starch was hydrolysed with M H_2SO_4 and estimated as TMSi-glucose.

Determination of polyuronide. Soluble polyuronide was determined in the 5 mM Pi pH 7.2 soluble fraction by an automated procedure based on the carbazole–H_2SO_4 method [1].

Fruit firmness was measured using a standard penetrometer with an 8 mm plunger.

Acknowledgements—I am indebted to Dr. M. Knee for his advice and to Mr. P. Henderson for his help with this work.

REFERENCES

1. Knee, M. (1973) *Phytochemistry* **12,** 1543.
2. Bartley, I. M. (1974) *Phytochemistry* **13,** 2107.
3. Talmadge, K. W., Keegstra, K., Bauer, W. D. and Albersheim, P. (1973) *Plant Physiol.* **51,** 158.
4. Loescher, W. H. and Nevins, D. J. (1972) *Plant Physiol.* **50,** 556.
5. Loescher, W. H. and Nevins, D. J. (1973) *Plant Physiol.* **52,** 248.
6. Kertesz, Z. I., Eucare, M. and Fox, G. (1959) *Food Res.* **24,** 14.
7. Knee, M. (1975) *Phytochemistry* **14,** 2181.
8. Knee, M. (1975) *Phytochemistry* **14,** 2213.
9. Dunn, G. (1974) *Phytochemistry* **13,** 1341.