2. Ultra-violet irradiation of the cells suppressed the dehydrogenating activity. Dehydrogenases for acids were more affected than those for alcohols and sugars.

3. Hypertonic NaCl solutions inhibited the dehydrogenating mechanisms for almost all donators, hypotonic ones slightly accelerated them. Only formic dehydrogenase was nearly insensitive to osmotic changes.

4. Repeated freezing and thawing inhibited the activity of almost all dehydrogenases tested, but

formic dehydrogenase remained uninfluenced by as many as ten freezings. The diminution of the dehydrogenating velocity was not due to the dilution of a coenzyme.

5. Cell-free extracts, prepared by centrifugation of autolyzed bacteria, contained lactic acid dehydrogenase.

6. It is concluded that most dehydrogenating systems are in some way linked to the cell structure. Only formic and lactic dehydrogenases are in some degree independent of the intactness of cell structure.

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The Determination of Starch in Apple Tissue

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In the determination of starch present in apple tissues by the use of an enzyme, the tissue must first be obtained in a finely divided state so that the starch can be easily extracted and hydrolyzed. It is noticeable in the literature on starch determinations in apples that difficulty is experienced in obtaining complete extraction of starch, and that different values may be obtained according to the method of extraction and the enzyme which is used for hydrolysis.

In 1926 Gerhardt was able to extract starch quantitatively with boiling water from the ethanol-insoluble residue of dried apple tissue after removal of the substances soluble in 10% ethanol.

Widdowson (1932) records that she was unable to extract all the starch from apple tissue, unless the ethanol-insoluble residue was previously extracted, with a 1% K-oxalate solution, to remove pectin. Even after this treatment the residue had to be thoroughly ground in a mortar before complete hydrolysis of the starch by takadiastase could be effected.

Denny (1934) found it necessary to extract the ethanol-insoluble residue several times with ethyl ether and then to grind the dried tissue to a powder. After grinding the powder with water in a mortar he was able to determine starch quantitatively with takadiastase. He also describes a method based on the preliminary extraction of the starch in hot concentrated CaCl₂ solution, which, he states, gave true values for starch.

In 1936 Hanes determined starch in ethanol-extracted apple tissues by first rendering the fibre soluble by treatment with ethanolic HCl, and then hydrolyzing with β -amylase, which is specific for starch. His starch determinations were made on a set of ten apple residues supplied by Dr Kidd from an experiment with Bramley's apples, for which the authors had already obtained values by hydrolysis with takadiastase. Comparison of the results suggested the presence of a fraction hydrolyzable by takadiastase, but not by β -amylase.

The authors have not experienced difficulty in carrying out complete hydrolysis with takadiastase;

this may be due to the pre-treatment of the apples. The present investigation was carried out during 1936-7 in order to make further comparison of the starch values obtained by hydrolysis with takadiastase and β -amylase.

METHODS

The apples used were four pickings of Worcester Pearmain which were gathered on (i) 18 July, (ii) 29 July, (iii) 15 August and (iv) 2 September 1935, and stored at 10°, and which formed part of an experiment conducted by Drs Kidd and West in this laboratory. All samples of fruit for analysis were frozen to -20°, and then ground in the frozen state to a fine powder, which was weighed out in samples of 100 g. and kept at -20° in covered jars until required. The powder was extracted for 4 hr. with boiling 80% ethanol, and the insoluble residue dried to constant weight at 100°. The residue was then in a finely divided condition which crumbled to a fine powder when rubbed between the fingers.

Starch was determined in the ethanol-insoluble residue by the following methods.

Method 1. Ethanol-insoluble residue treated with takadiastase. This method is based on Widdowson's method (1932), in which the samples of the insoluble residue were digested with water in a boiling water-bath, and the starch hydrolyzed with takadiastase. Reducing sugar was determined by Maskell's (1935) modification of Shaffer & Hartman's (1921) micro-method, and related to the reducing power of a pure hydrolyzed apple starch. A blank was carried out on the takadiastase solution alone.

Method 2. Ethanol-insoluble residue shaken with 1% (w/v) potassium oxalate solution, and then hydrolyzed with takadiastase. The insoluble residue was shaken with 1% K-oxalate solution for 5 hr. in a mechanical shaker as described by Widdowson (1932). The residue was then filtered and washed free from oxalate with cold water, and then hydrolyzed with takadiastase as in method 1. The K-oxalate solutions were all tested with I₂ solution, and any colour reactions were recorded.

Method 3. Ethanol-insoluble residue hydrolyzed with β -amylase. The β -amylase was kindly supplied by Dr C. S. Hanes. The ethanol-insoluble residue was rendered soluble by treatment with ethanolic HCl, and then hydrolyzed with β -amylase, as described by Hanes (1936). The reducing power of the hydrolyzed starch was determined by the same method as in (1).

RESULTS

The values obtained by these methods are shown in Table 1, together with the colours produced by treating the K-oxalate extracts with I₂ solution in method 2.

DISCUSSION

Values for starch obtained by methods 2 and 3 show good agreement, but values by method 1 are much higher. This indicates that takadiastase hydrolyzes a substance or substances other than starch in the residue. The K-oxalate pre-treatment evidently removes this substance or substances, which may be called the 'non-starch' fraction.

When the K-oxalate filtrates were tested with I₂ solution the colours changed from deep blue, in the samples stored only a few days, to mauve or red-brown, in samples stored approximately 15 days; after 15 days no colour was obtained. A similar result was obtained by shaking the residue with cold water. Hydrolysis of the aqueous filtrate with takadiastase gave a reducing substance. Pure apple starch subjected to the same treatment with K-oxalate did not give any colour with I₂ solution. The 'non-starch' fraction, extractable with cold water or 1% K-oxalate solution, is therefore responsible for the colour reaction with I₂, and suggests the presence of dextrans.

Denny (1934) determined starch in plant tissues by takadiastase as described in method 1, and compared results with those obtained by extracting the starch with hot concentrated CaCl₂ solution, precipitating the starch with I₂, removing the I₂, and hydrolyzing the purified starch with takadiastase. He found that the first method gave values higher than the second, and assumed that the latter gave the true starch. He called the difference between the values obtained by the two methods 'the non-starch fraction hydrolyzable by takadiastase', but did not identify this fraction.

Widdowson (1932) examined the ethanol-insoluble residue from Bramley's Seedling apples for dextrin, by extracting with water at 38° for 24 hr., filtering, and treating the filtrate with I₂ solution, but did not obtain any colour. She also hydrolyzed the filtrate with takadiastase, but did not obtain any appreciable reducing activity, and therefore concluded that there is never more than a trace of dextrin in Bramley's apples. It is suggested that Widdowson's failure to obtain any coloration with I₂ may be due to the same cause that prevented complete extraction of the starch with water, namely, that the fibre was not in a sufficiently disintegrated condition to permit the penetration of the solvent. It appears that when the apple is frozen and ground, and kept frozen at -20°, the material of the cell wall may be broken down by freezing, and consequently the ethanol-insoluble residue is more permeable to the action of the solvent.

Gerhardt (1926) was able to extract from 0.1 to 0.59% of dextrin from apple tissue. He dried the apple tissue in a partial vacuum (50 mm. pressure) at 70° and ground the dried tissue in a mill until the powder would pass through a 100-mesh sieve. After removal of sugars, he extracted the residue with 10% ethanol at 50° for 30 min., evaporated the ethanol, clarified the solution, hydrolyzed with 2.5% HCl (approx. 0.7N [?]), and calculated the reducing substances as dextrans.

Hence, there is some evidence that dextrans are present in apples at certain stages of growth.

Table 1. *Starch determinations on four pickings of Worcester Pearmain apples*

(Results as g./100 g. of fresh weight.)

Date of picking	Average size of fruit (g.)	Days in store (at 10°)	Hydrolysis by			Colour obtained by addition of I ₂ solution to the K-oxalate extract.
			Takadiastase (g./100 g.)	Takadiastase after extraction with K-oxalate (g./100 g.)	β-amylase (g./100 g.)	
18. vii. 35	25.6	0	2.09	1.92	—	Blue
		2	2.00	1.72	—	"
		4	1.73	1.66	—	Mauve
		4	1.54	1.42	—	"
		8	1.38	1.08	—	Red-brown
		12	0.97	0.79	—	None
		20	0.23	0.10	—	"
		20	0.26	0.10	—	"
		30	0.16	0.05	—	"
		40	0.16	0.05	—	"
		50	0.12	0.04	—	"
		60	0.17	0.04	—	"
		(at 10°)				
		0	2.26	2.09	—	Blue
		2	2.20	1.98	—	"
		4	2.00	1.74	—	"
		4	1.70	1.59	—	"
		8	1.29	1.21	—	None
29. vii. 35	37.2	12	0.79	0.65	—	"
		19	0.22	0.08	—	"
		19	0.19	0.07	—	"
		30	0.15	0.03	—	"
		40	0.16	0.02	—	"
		50	0.15	0.02	—	"
		60	0.17	0.02	—	"
		(at 10°)				
		0	2.48	2.17	2.14	Blue
		2	2.58	2.11	2.23	"
		5	2.05	1.66	1.69	"
		8	1.49	1.07	1.19	"
		11	0.90	0.65	0.62	"
15. viii. 35	61.8	14	0.64	0.43	0.38	Mauve
		18	0.31	0.15	0.13	None
		21	0.26	0.08	0.07	"
		25	0.19	0.03	0.03	"
		28	0.17	0.03	0.03	"
		32	0.17	0.02	0.03	"
		65	0.17	0.02	0.02	"
		127	0.17	0.02	0.02	"
		(at 1°)				
		0	2.48	2.17	2.14	Blue
		5	2.10	1.81	1.72	"
		11	1.55	1.19	1.19	"
		16	1.22	0.88	0.94	"
15. viii. 35	61.8	22	0.83	0.58	0.54	Mauve
		28	0.29	0.11	0.11	None
		34	0.48	0.25	0.25	"
		40	0.33	0.17	0.15	"
		46	0.22	0.08	0.06	"
		51	0.23	0.04	0.05	"
		56	0.19	0.03	0.03	"
		63	0.20	0.03	0.02	"
		69	0.18	0.02	0.03	"
		204	0.16	0.02	0.03	"
		(at 10°)				
		0	1.79	1.52	—	Blue
		2	1.71	1.53	—	"
2. ix. 35	72.4	4	1.39	1.22	—	"
		4	1.52	1.19	—	"
		8	0.72	0.60	—	Mauve
		12	0.41	0.31	—	None
		19	0.16	0.06	—	"
		19	0.14	0.06	—	"
		30	0.09	0.03	—	"
		40	0.10	0.04	—	"
		51	0.06	0.02	—	"
		60	0.09	0.02	—	"

Fig. 1. Changes in the non-starch fraction of Worcester Pearmain apples. A. With growth from 18. vii. 35 to 2. ix. 35. B. First pick gathered on 18. vii. 35 and stored at 10°. C. Second pick gathered on 29. vii. 35 and stored at 10°. D. Third pick gathered on 15. viii. 35 and stored at 10° ●—●, and at 1° ○—○. E. Fourth pick gathered on 2. ix. 35 and stored at 10°.

The difference in the values obtained by methods 1 and 2 (or 3) is a measure of the 'non-starch'

fraction, and Fig. 1 shows the changes in this fraction during growth and storage.

This fraction, at the time of picking, increases in value up to 15 August, and then shows a slight fall on 2 September, which coincides with the increase and decrease of true starch. During the first few days of storage, there is a slight increase in 'non-starch' in the first and second picks, a definite increase in the third pick, but an immediate fall in the fourth pick. In each gathering, the non-starch fraction falls to an approximately constant value after the disappearance of true starch.

As there is no increase in true starch during storage, it is possible that the 'non-starch' is a degradation product. The greatest increase in 'non-starch' is found where true starch is at its maximum, that is, on 15 August.

SUMMARY

1. The freezing of apple tissue produces a fibre in which all the starch can be hydrolyzed with takadiastase.

2. If the ethanol-insoluble residue from frozen apple is shaken with 1 % potassium oxalate solution, a 'non-starch' fraction hydrolyzable by takadiastase is extracted. The evidence suggests the possibility of the presence of dextrins.

3. The starch determinations by Hanes's method using β -amylase give similar values to those obtained by the action of takadiastase on the residue after treatment with potassium oxalate. These values are assumed to be true starch.

4. The 'non-starch' fraction falls during storage to an approximately constant figure. The fall takes place during the period of true starch hydrolysis, and the substances giving colours with iodine are also present in the fraction during this period.

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