LII. THE SPECIFIC ACTION OF PLANT FERMENTS.

I. THE SPECIFIC CONDITIONS OF ACTION OF LEAF INVERTASES.

BY A. V. BLAGOVESCHENSKI AND N. I. SOSSIEDOV.

From the Laboratory of Plant Physiology, State University, Tashkent.

(Received March 11th, 1925.)

THE investigations of Sørensen [1909], Michaelis and Davidsohn [1911] and Euler and Emberg [1919] on the optimal p_H -values for the action of yeast-invertases, and those of Euler and Svanberg [1921] for the action of intestinal invertase of man, gave a distinct dissimilarity of these values: the greatest action of yeast-invertase was found at p_H 3.5–5.5; that of intestinal invertase at p_H 5–7. That the invertases from various yeasts are not identical is made clear by Willstätter and Kuhn [1923].

Our experiments on the invertases of leaves of various plants give a new confirmation for the Willstätter point of view, according to which we have as many invertases as sources from which they are obtained.

We investigated the water extracts from air-dried powdered leaves of 14 species of wild and cultivated plants. The leaves were taken under identical conditions from various individuals of the same species (July–August, 1924), dried rapidly at 25–30° and ground in a mortar. 1 g. of a leaf-powder was mixed with 60 cc. of water and filtered through a dry filter after standing at 25° during 24 hours with toluene. 5 cc. of clear filtrate were mixed with 10 cc. of phosphate buffer mixture and 5 cc. 8 % saccharose solution. To all experimental flasks toluene was added and they were kept at 25°. The determinations of invert-sugar were made by the Benedict-Lewis picric acid method. The standard solutions were prepared each week according to Willaman and Davison's [1924] directions, with intervals of 0.125 mg. of invert-sugar. For each determination 0.5 cc. of the solution to be investigated was taken. The duration of each single experimental series varied from 23 to 91 hours at about 30°.

Exp. 1. The leaves of *Prangos pabularia* Lindl., a species of the Umbelliferae indigenous to the valleys of West Tian-Shan, were investigated at the Botanical Station of the University of Chimgan. The initial content of reducing sugar in 0.5 cc. of the experimental solution is 1.0 mg.; duration of hydrolysis 47½ hours.

pH	Reducing sugar mg. in 0.5 cc.	Change	
		mg.	%
4.5	3.50	+2.50	48
5.0	4.75	3.75	71
5.6	5.25	4.25	81
5.9	5.50	4.50	86
6.2	5.75	4.75	90
6.5	6.00	5.00	95
6.6	6.25	5.25	100
7.0	5.25	4.25	81

Exp. 2. The subterranean leaves of bulbs of *Ungernia Severzovii* (Rgl.) B. Fedtsch., a species of the Amaryllidaceae from West Tian-Shan. The initial content of reducing sugar in 0.5 cc. of the experimental solution is 1.125 mg.; duration of hydrolysis 49½ hours.

pH	Reducing sugar mg.	Change	
		mg.	%
4.5	1.425	0.300	91
5.0	1.450	0.325	100
5.6	1.425	0.300	91
5.9	1.300	0.125	39
6.2	1.250	0.100	33
6.5	1.150	0.025	9
6.6	1.125	0.000	0

Exp. 3. The leaves of *Juglans fallax* Dode, from the Chimgan valley in West Tian-Shan. The initial content of reducing sugar is 0.650 mg.; duration of hydrolysis 46½ hours.

pH	Reducing sugar mg.	Change	
		mg.	%
4.5	3.750	3.100	55
5.0	4.250	3.600	64
5.6	5.250	4.600	82
5.9	6.250	5.600	100
6.2	5.750	5.100	91
6.5	5.000	4.350	78
6.6	3.750	3.100	55
7.0	2.750	2.100	38

Exp. 4. The leaves of *Vitis vinifera* L. cultivated at Tashkent. The initial content of reducing sugar in 0.5 cc. of the experimental solution is 0.525 mg.; duration of hydrolysis 48 hours.

pH	Reducing sugar mg.	Change	
		mg.	%
4.4	1.150	0.625	64
4.5	1.450	0.925	95
5.0	1.500	0.975	100
5.6	1.375	0.850	87
5.9	1.375	0.850	87
6.2	1.225	0.700	72
6.5	1.100	0.575	59
6.6	0.475	0.475	49
7.0	0.525	0.000	0

Exp. 5. The leaves of *Gossypium herbaceum* L. from the Turkestan Plant Breeding Station at Tashkent. The initial content of reducing sugar is 0.375 mg.; duration of hydrolysis 41 hours.

p_H	Reducing sugar mg.	Change	
		mg.	%
4.0	0.650	0.275	31
4.4	0.800	0.425	49
4.5	1.150	0.775	89
5.0	1.150	0.775	89
5.6	1.250	0.875	100
5.9	1.175	0.800	91
6.2	1.050	0.675	77
6.5	0.975	0.600	69
6.6	0.750	0.375	43
7.0	0.800	0.425	49

Exp. 6. The leaves of *Gossypium hirsutum* L. from the Turkestan Breeding Station at Tashkent (strains No. 182 and No. 509). The initial content of reducing sugar for No. 182 is 0.875 mg. and for No. 509 1.100 mg.; duration of hydrolysis for No. 182 46½ hours and for No. 509 45 hours 50 minutes. For p_H 4.0 and 4.4 glycine buffer solutions were used.

No. 182				No. 509			
p_H	Reducing sugar mg.	Change		Reducing sugar mg.	Change		
		mg.	%		mg.	%	
4.0	0.875	0.0	0	1.100	0.0	0	
4.4	0.875	0.0	0	1.100	0.0	0	
4.5	1.150	0.275	79	1.600	0.500	91	
5.0	1.225	0.350	100	1.650	0.550	100	
5.6	1.200	0.325	93	1.500	0.400	73	
5.9	1.175	0.300	86	1.450	0.350	64	
6.2	1.125	0.250	71	1.375	0.275	50	
6.5	1.100	0.225	64	1.250	0.150	27	
6.6	1.000	0.125	36	1.175	0.075	18	
7.0	0.950	0.075	22	1.225	0.125	23	

Exp. 7. The leaves of *Glycyrrhiza glabra* L. from Chimgan. The initial content of reducing sugar is 0.500 mg.; duration of hydrolysis 91 hours.

p_H	Reducing sugars mg.	Change	
		mg.	%
4.0	0.500	0.0	0
4.4	0.500	0.0	0
4.5	0.900	0.400	100
5.0	0.850	0.350	88
5.6	0.775	0.275	69
5.9	0.600	0.100	25
6.2	0.525	0.025	6
6.5	0.500	0.0	0

Exp. 8. The leaves of *Platanus orientalis* L. from the University Botanical Garden at Tashkent. The initial content of reducing sugar is 0.625 mg.; duration of hydrolysis 47 hours 45 minutes. For p_H values from 3.3–4.0 glycine buffer solutions were used.

p_H	Reducing sugar mg.	Change	
		mg.	%
3.3	0.925	0.300	34
3.7	1.250	0.625	72
4.0	1.250	0.625	72
4.5	1.500	0.875	100
5.0	1.425	0.800	92
5.6	1.375	0.750	86
6.2	1.250	0.625	72
6.6	0.900	0.275	32
7.0	0.875	0.250	29

Exp. 9. The leaves of *Pyrus communis* L. (Tashkent). The initial content of reducing sugar is 0.550 mg.; duration of hydrolysis 39 hours.

p_{H}	Reducing sugar mg.	Change	
		mg.	%
4.5	2.250	1.700	60
5.0	2.375	1.825	65
5.6	2.750	2.200	78
5.9	3.200	2.650	94
6.2	3.375	2.825	100
6.5	3.000	2.450	87
6.6	1.825	1.275	45
7.0	1.425	0.875	31

Exp. 10. The leaves of *Pyrus malus* L. (Tashkent). The initial content of reducing sugar is 0.825 mg.; duration of hydrolysis 49½ hours.

p_{H}	Reducing sugar mg.	Change	
		mg.	%
4.4	4.750	3.925	72
4.5	5.875	5.050	93
5.0	6.160	5.325	98
5.6	6.250	5.425	100
5.9	5.950	5.125	95
6.2	5.500	4.675	86
6.5	4.625	3.800	70
6.6	3.500	2.675	49
7.0	2.625	1.800	33

Exp. 11. The leaves of *Quercus pedunculata* L. (Tashkent). The initial content of reducing sugar is 0.775 mg.; duration of hydrolysis 23 hours.

p_{H}	Reducing sugar mg.	Change	
		mg.	%
4.5	5.250	4.475	88
5.0	5.375	4.600	90
5.6	5.500	4.725	93
5.9	5.625	4.850	95
6.2	5.750	4.975	98
6.5	5.875	5.100	100
6.6	5.375	4.600	90
7.0	2.625	1.850	58

Exp. 12. The leaves of *Syringa vulgaris* L. (Tashkent). The initial content of reducing sugar is 0.675 mg.; duration of hydrolysis 38½ hours.

p_{H}	Reducing sugar mg.	Change	
		mg.	%
4.5	1.075	0.400	89
5.0	1.025	0.350	76
5.6	1.025	0.350	76
5.9	1.050	0.375	83
6.2	1.125	0.450	100
6.5	1.050	0.375	83
6.6	1.075	0.400	89
7.0	0.950	0.275	61

Exp. 13. The leaves of *Fraxinus* sp. (Tashkent). The initial content of reducing sugar is 0.350 mg.; duration of hydrolysis 62 hours.

p_H	Reducing sugar mg.	Change	
		mg.	%
4.5	1.450	1.100	92
5.0	1.475	1.125	94
5.6	1.500	1.150	96
5.9	1.500	1.150	96
6.2	1.550	1.200	100
6.5	1.500	1.150	96
6.6	1.300	0.950	79
7.0	1.200	0.850	71

Exp. 14. Finally, we investigated the expressed juice of *Salicornia herbacea* L. (from the salt desert Golodnaja Steppe). No reducing sugar at start; duration of hydrolysis $64\frac{1}{2}$ hours.

p_H	Reducing sugar mg.	Change	
		mg.	%
4.5	0.950	0.950	68
5.9	1.200	1.200	86
6.2	1.400	1.400	100
6.5	1.250	1.250	90
6.6	1.175	1.175	84
6.8	1.125	1.125	80
7.0	1.025	1.025	73
7.2	0.975	0.975	70
7.4	0.875	0.875	63
7.7	0.925	0.925	66
7.9	0.800	0.800	57
8.0	0.625	0.625	45

All our experiments give a definite conclusion that a definite optimal hydrogen ion concentration exists for each plant species and that variation of ratio of hydrolysis with change in p_H is of a very specific character.

REFERENCES.

- Euler and Emberg (1919). *Z. Biol.* **69**, 349.
 Euler and Svanberg (1921). *Z. physiol. Chem.* **115**, 43.
 Michaelis and Davidsohn (1911). *Biochem. Z.* **35**, 386.
 Sørensen (1909). *Biochem. Z.* **21**, 131.
 Willaman and Davison (1924). *J. Agric. Res.* **28**, 479.
 Willstätter and Kuhn (1923). *Z. physiol. Chem.* **125**, 32.