INULIN CONTAINING PLANTS AS A SOURCE OF ETHYL ALCOHOL—THE ACTION OF INVERTASE FROM YEAST

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Just as there exists in nature a large group of plants which store starch, we have another large group which store inulin, a polysaccharide somewhat similar to starch but yielding, upon complete hydrolysis, fructose instead of glucose. The best known representatives are the Jerusalem artichoke, dahlia, and chicory. The yields per acre of some of these plants are great enough that they may be considered as potential competitors of present sugar sources. While ordinary brewer's yeast apparently will not attack pure inulin, Windisch,[14] Rudiger[10] and Vadas[13] have reported good yields of alcohol from artichoke tubers without any preliminary hydrolysis. Vadas stresses the fact that if the tubers are cooked, some of the raw pulp should be included to furnish inulase or other enzymes which function in breaking down the complex polysaccharides in the same way that malt breaks down starch. Underkofler, McPherson and Fulmer[12] have recently studied the fermentation of dried artichoke material and report excellent results without a preliminary hydrolysis. Similar findings had been made by Appleman[1] in this laboratory and the work has since been confirmed and extended by Clark.[2]

As early as 1867 Dubrunfaut[5] presented data indicating the presence of sucrose in plants containing inulin. The degradation, or change to simpler nonreducing substances, in the roots or tubers which have been allowed to remain in the ground over winter has been studied by Geslin and Wolf,[6] Colin,[3] Thaysen Baker and Green and De Vito.[4] Evidence for the presence of sucrose is (a) the increase in the reducing sugar as a result of the action of invertase, (b) the lowering of the rotation after hydrolysis, and (c) the presence of aldose sugars as shown by their oxidation by iodine in alkaline solution. However, the actual isolation of the sugar and positive proof of its occurrence seem to be lacking. There is considerable controversy among the workers as to the nature of the materials which may accompany the apparent sucrose.

Before attempting to utilize plants containing inulin as a source of alcohol it is desirable to know as much as possible about the properties of their carbohydrates, particularly as regards their response to the enzymes which occur in yeast.

It was the object of this work, which is preliminary in character, to study the action of invertase as ordinarily applied to the quantitative estimation of sucrose in plant materials and to compare the results with those obtained by the alternate acid hydrolytic method.

Experimental

Plant materials.—The products subjected to analysis were samples of artichoke, chicory and dahlia roots. All were harvested in the late fall and had been washed, sliced in ⅛ inch slices, and dried in a well ventilated oven at about 160°C.

Invertase.—The enzyme was obtained from baker's yeast according to the procedure outlined by Morrow.[9] The product was purified by dialysis in a collodion bag.

Analytical procedure.—The thoroughly dried samples were finely ground in a drug mill. A five-gram portion was weighed into a 400 ml. beaker, 150 ml. of hot distilled water added, the beaker placed on a steam cone and heated for thirty minutes. When the heating period was complete the mixture was filtered, the residue washed, and the filtrate cooled. Neutral lead acetate (8 per cent solution) was added to precipitate the proteins, the mixture was made up to 500 ml. and filtered. To the filtrate enough solid disodium phosphate was added to precipitate the excess lead and the solution was again filtered.

TABLE 1.—APPARENT SUCROSE IN PLANTS CONTAINING INULIN—ANALYSIS OF THE EXTRACT OF THE DRIED MATERIAL

Plant Material	Per cent Apparent Invert Sugar			Total Available Reducing Sugar (Complete Hydrolysis)
	Initially Present	After Invertase Hydrolysis	After Cold HCl Hydrolysis	
Artichoke	1.76	39.1	48.0	59.2
	1.01	25.3	31.8	
	2.00	33.3	37.8	
Chicory	4.10	34.9	48.2	48.4
	4.66	29.4	46.6	
	6.58	37.8	46.9	
Dahlia	6.11	26.7	42.6	50.7
	6.20	34.0	41.7	
	6.54	26.6	42.9	

Per cent Apparent Sucrose (Average)

	By Invertase Hydrolysis	By HCl Hydrolysis	Per cent Sucrose by Invertase
			Total Sugar
Artichoke	31.0	37.6	52.5
Chicory	33.2	42.1	68.5
Dahlia	23.0	36.1	45.3

Aliquots of the filtrate were analyzed for reducing sugar by the Munson and Walker method. Other portions were treated for hydrolysis of sucrose by (a) invertase and (b) HCl at room temperature and analyzed according to official methods.[8] The amount of reducing sugar after hydrolysis was estimated by the Lane and Eynon method. From the increase, the apparent sucrose was calculated for the invertase procedure. The total available sugar was established by hydrolyzing at 70° with acid according to the specifications of Jackson and associates[7] and following with a Lane and Eynon titration.

The results are shown in Table 1.

Discussion.—The higher values for apparent sucrose by the acid as compared to the invertase hydrolysis were anticipated. Close agreement of the results from the two methods of treatment would be obtained only if sucrose were present alone or along with materials hydrolyzable only with difficulty. However, the variation in duplicate analyses by the invertase method at first appeared disconcerting. Since the conditions observed for the reaction were adequate for inversion of a quantity of sucrose far in excess of the total sample weight taken, one cannot assign the lack of agreement to incomplete hydrolysis of this sugar. The results suggest that the invertase, as ordinarily prepared, may contain carbohydrases other than sucrose in small amounts which act slowly upon other materials present in the extract. Since repeat analyses made on different days were subject to widely varying temperature conditions (24 hours at room temperature), the extent of these other reactions must have varied considerably. As has been indicated by previous workers, the ready fermentability of the dried material and of the spring harvested crops probably results from a slight degradation of inulin to compounds more susceptible to the action of the yeast.

Entirely apart from the fermentation aspect it should be emphasized that if "invertase" does contain other carbohydrases, the assumption that the system is specific for the sucrose linkage may lead to considerable error when applied without discrimination to the determination of sucrose in plant products.

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