YIELD AND PURITY OF LEVULOSE DERIVED FROM THE CALCIUM LEVULATE PROCESS

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ABSTRACT

Calcium levulate, although relatively insoluble in water, is appreciably soluble in sugar solutions. The yield of levulose from sugar mixtures is therefore dependent upon the ratio of levulose to total sugar in the crude mixture. The principal nonlevulose sugars occurring in plant juices after hydrolysis of the polysaccharides are dextrose and the isomeric difructose anhydrides. As a by-product of hydrolysis fructosans may be introduced. Data are given which show how much levulate is held in solution by dextrose and by the difructose anhydrides. Increased temperature diminishes the yield of levulate markedly. Quantitative measurements were made which show the yield of levulate from various crude juices and the distribution of levulose, total reducing sugar, and lime among the waste water, wash, and levulate cake. The data show satisfactory product balances.

The levulate cake suspended in water and decomposed with carbon dioxide yielded sirups of purities fluctuating between 91 and 97 percent. It was ascertained that the sugar suffered a loss of purity during the transformation of calcium bicarbonate to carbonate, which imparted an alkaline reaction to the solution. In the final series of experiments calcium bicarbonate was removed from the cold suspension by additions of lime. From the resulting filtrate calcium was precipitated quantitatively by oxalic acid. All adjustments were made by means of conductometric measurement. By the modified method levulose sirups of 99 percent purity were obtained.

CONTENTS

I. INTRODUCTION

In a previous article Jackson, Silsbee, and Proffitt [1] described a process for the preparation of levulose from the juices of the jerusalem artichoke and other natural sources in which the levulose was isolated from the crude juice, after hydrolysis of the polysaccharides, by means of a modification of Dubrunfaut's calcium levulate method, the modification producing a readily filterable suspension instead of the difficultly filterable mixture of the original process. The levulose sirups obtained in a number of experiments showed purities which averaged 93.8 percent, but which fluctuated without explainable cause over the wide range from 91 to 97 percent. The predic-

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[1] BS Sci. Pap. 20, 587 (1926), S519.

tion was made [2] that "there are still some undetermined factors which upon further study will tend to produce the higher purities and yields more uniformly." In the present investigation we have sought to discover the influences which affect the yield of levulate, and particularly to modify the procedure in order to obtain higher purities in the final levulose sirups.

II. PRECIPITATION OF CALCIUM LEVULATE

In the article cited it was shown that calcium levulate could be caused to precipitate in a coarsely crystalline state, which permitted rapid filtration and washing, by making continuous additions of lime and levulose to a suspension of previously formed levulate, the newly formed levulate tending to deposit on nuclei already present, building them up in some instances to a length of 0.2 mm, and increasing their cross section correspondingly. This method was followed in the experimental work described in the present paper.

In applying the method to the miscellaneous materials from which levulose could be prepared it was observed that the yield of levulate was, as in the previous work, subject to wide fluctuations. In the earlier article it was shown that the solubility of calcium levulate was increased by dissolved dextrose or by an excess of levulose. Experiment showed that the precipitate was also soluble in sucrose solutions. It then appeared probable that calcium levulate was more or less dissolved by any sugar which occurred in the product serving as the source of levulose and that the presence of nonlevulose sugars was mainly responsible for the fluctuating yields.

The problem then was to identify the nonlevulose sugars which occurred in the juices and to measure their effect on the solubility of calcium levulate. The present discussion is concerned particularly with artificial mixtures of pure sugars and with the juices of the jerusalem artichoke, dahlia, and chicory. It will be assumed that the polysaccharides in the plant juices have been hydrolyzed, preferably by sulphuric acid, and the juices defecated after cooling to room temperature by addition of milk of lime to pH 7 to 8.

The principal nonlevulose sugars which occur in such plant juices are dextrose, which invariably accompanies levulose in natural products, and the three isomeric difructose anhydrides [3] which are always present in the products of the acid hydrolysis of inulin. Conceivably other sugars may occur, but probably in small amount.

In addition to the sugars mentioned, whose presence is unavoidable, others may be present which are introduced during hydrolysis and defecation. If the hydrolysis reaction is prolonged levulose suffers the loss of 1 molecule of water and changes to the nonreducing fructosans first isolated by Pictet and Chavan,[4] and designated by them heterolevulosan and its dimer, diheterolevulosan. Mathews and Jackson [5] have shown that this dehydration occurs not only in the extreme acidity employed by Pictet and Chavan, but in the more mildly acid solutions required for the hydrolysis of inulin. These fructosans are therefore present in all juices in which the polysaccha-

    [2] See p. 612 of reference 1.
    [3] Jackson and Goergen, BS J. Research 3, 27 (1929) RP79; Jackson and McDonald, BS J. Research 6, 709 (1931) RP299.
    [4] Helv. Chim. Acta 9, 809 (1926).
    [5] BS J. Research 11, 627 (1933) RP611.

rides are hydrolyzed by acid, but their amount can be kept at a minimum by cooling the acidified solution immediately upon the completion of the hydrolysis.

Incomplete hydrolysis, on the other hand, leaves soluble products which not only constitute a loss of potential levulose, but by their solvent effect on calcium levulate cause a further loss of levulose, which, but for their presence, could be avoided. To avoid excessive or deficient conversion a careful control of the hydrolysis reaction is therefore of the first importance.

If after completion of the hydrolysis the defecation is carried out in too highly alkaline solution, or if the temperature is too high, or if as a result of inadequate agitation local high alkalinities occur, levulose may undergo the Lobry de Bruyn-van Ekenstein transformation to dextrose and mannose, resulting in a loss of levulose by decomposition and by the solvent effect of the products of the decomposition on calcium levulate.

The plant juices from which levulose can be obtained usually have a total sugar purity of about 85 percent. Conceivably the nonsugars have a solvent effect upon calcium levulate, but thus far it has proved difficult to separate them from the sugars and no attempt has been made to measure their influence.

Since dextrose is in most instances the predominating impurity its solvent effect on calcium levulate was studied in detail. The experiments were conducted in a metal container holding about 1,800 ml, which was immersed in a large water bath whose temperature was controlled by manual operation. The container was half filled with water, and alternate additions were made of lime and invert sugar in the manner previously described until the container was filled. About one-half of the resulting suspension of levulate was filtered and the filtrate or "waste water" subjected to analysis.[6] The container was again filled by adding reactants to the remaining portion of the levulate suspension and again half was filtered for analysis of the waste water. This process was repeated 4 or 5 times at each of three temperatures. There resulted in each series an accumulation of dextrose.

The experimental results are given in table 1, which shows the composition of the waste waters saturated with calcium levulate. The amount of levulose held in solution as levulate varies with the concentration of dextrose, although not linearly, since the ratio of levulose to dextrose is not constant at all concentrations of sugar. It will be found significant to evaluate these ratios as well as the concentrations of sugar in the succeeding sections of this paper, but they will be expressed as ratios of levulose to total reducing sugar rather than as levulose to dextrose.

At each temperature the ratios of levulose to total reducing sugar are relatively higher in the more dilute solutions of dextrose and approach constant low values as the concentration of dextrose increases. Calcium levulate has an appreciable solubility in water and the solvent effect of dextrose is superimposed upon this. At the higher concentrations of dextrose the latter becomes the predominating effect.

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[6] For analytical methods see BS J. Research **8**, 403 (1932) RP426; **9**, 597 (1932) RP495.

TABLE 1.—*Composition of waste water from calcium-levulate precipitate from invert sugar, showing effect of dextrose and of temperature*

Temperature	Reducing sugar	Levulose	Dextrose by difference	Ratio, levulose to reducing sugar	CaO	Ratio, CaO to reducing sugar
°C	g/100 ml	g/100 ml	g/100 ml		g/100 ml	
5	1.587	0.289	1.298	0.182	0.375	0.236
	2.642	.362	2.280	.147	.585	.221
	3.670	.477	3.193	.130	.784	.188
	4.772	.616	4.156	.129	.998	.209
10	1.830	.392	1.438	.214	.393	.215
	3.142	.597	2.545	.190	.605	.192
	4.464	.795	3.669	.178	.850	.190
	5.190	.830	4.360	.160	1.076	.207
	6.262	.996	5.266	.159	1.311	.209
15	1.555	.428	1.127	.275	.347	.223
	3.028	.787	2.241	.260	.565	.187
	4.297	.984	3.313	.229	.821	.191
	5.269	1.175	4.094	.223	1.041	.198
	6.421	1.458	4.963	.227	1.297	.202

Since, as appears from the tabulated values, the lowest ratios in the waste water occur at the highest concentrations of dextrose, the greater yields of precipitated levulate from a given mixture of sugars are obtainable at the higher concentrations of reactants.

The effect of elevated temperature is a considerable increase in the solubility of the levulate, a 10° C rise causing a 75-percent increase in the ratio at its constant low value.

For later reference it will prove of interest to compute by extrapolation that at the lowest concentration of total sugar the ratio at 0° C would be about 0.15, and that under the same conditions a weight of levulose equal roughly to one-fifth of the weight of dextrose remains in solution.

The alkalinity of the waste water is directly proportional to the concentration of total reducing sugar. Independently of temperature 1 g of total sugar holds in solution about 0.2 g of CaO (column 7, table 1). The lime in the waste water is derived not alone from the calcium levulate, but is also dissolved by the water and dextrose.

In table 2 is shown the effect of temperature upon the yield of levulose from artichoke juices in which the ratio of levulose to total reducing sugar was 76.8 percent. The data show that the yield diminishes very rapidly as temperature is increased.

TABLE 2.—*Effect of temperature of reaction upon yield of levulose*

[Hydrolyzed artichoke juices. Ratio, levulose to total reducing sugar, 76.8 percent]

Precipitation temperature	Reaction mixture			Levulate cake		Yield of levulose
	Weight	Reducing sugar	Levulose	Weight	Levulose	
°C	g	%	%	g	g	%	%
3.2	1,855	7.33	5.63	104.4	444	19.57	86.9	82.3
10.7	1,800	7.16	5.50	99.0	328	22.98	75.4	76.2
16.0	1,496	7.03	5.40	80.8	249	20.75	51.7	64.0

The solvent effect of the isomeric difructose anhydrides on calcium levulate is illustrated by the analysis of the waste water from the precipitation of levulate at 0° C from pure inulin which had been hydrolyzed by sulphuric acid, the latter being subsequently removed as barium sulphate. The main purpose of the experiment was the estimation and isolation of the difructose anhydrides. The waste water was not analyzed directly but was subjected to a preliminary evaporation. Nevertheless, the pertinent ratios remained unaltered by this treatment.

The waste water from the levulate was exactly neutralized with oxalic acid and the filtered solution evaporated to about 10° Brix. Total solids were determined densimetrically, the total reducing sugars and levulose by the respective copper reduction methods, and the difructose anhydrides by difference. The evaporated solution contained in 100 ml 10.685 g solids, 5.260 g total reducing sugar, of which 3.351 g was levulose, and by difference 5.425 g of difructose anhydrides. The ratio of levulose to total reducing sugar was 0.64, a quantity far too great to be ascribed to the dextrose, as can be seen by reference to table 1. The excessive quantity of levulose is evidently held in solution by the difructose anhydrides. Previous to the discovery of these sugars the high ratio of levulose in the waste water from hydrolyzed inulin was unexplained. If the weight of difructose anhydrides is added to the weight of reducing sugar the computed ratio of levulose to total sugar proves to be 0.314, which indicates that a higher solvent effect is to be ascribed to the anhydro sugars than to dextrose. In crude juices no analytical method for the determination of the difructose anhydrides is available, but a departure of the ratios of levulose to total reducing sugar from those given in table 1 is an indication of their presence or of course the presence of other sugars not detectable by analysis.

In a few experiments on artichoke juices the action of the sulphuric acid was accidentally prolonged after the completion of the hydrolysis of the polysaccharides. The value of the ratio of levulose to reducing sugar in the waste water from the levulate precipitation rose to 0.58 in one instance, showing that the fructosans of Pictet and Chavan had been formed in considerable quantity by the action of the acid.

In table 3 are shown the results of systematic experiments on pure sugars and plant juices in which each of the four products, namely, the reaction mixture, waste water, wash, and levulate cake, was weighed and analyzed. The reaction mixture was prepared in the usual manner, sampled for analysis, and finally weighed. It was then filtered on a Büchner funnel and the waste water collected in a tared filter flask. When the waste water had drained to the surface of the levulate cake the filter flask was replaced by a second one for collection of the wash. In some instances the wash water (that is, the pure water used for washing) was weighed, in others its weight was determined by difference, the latter weights being indicated in the table by brackets.

Each of the four products was analyzed for the three constituents, levulose, total reducing sugar, and lime. The percent concentrations shown by the analyses are given in the left of the subdivided columns in table 3. Although these analytical data were obtained in small-

TABLE 3.—*Quantitative data on calcium levulate precipitation*

Line	Item	1 Pure sugars 0.0		2 Artichokes 1.0		3 Artichokes 1.5		4 Artichokes 3.0		5 Artichokes 0.5		6 Chicory 3.0		7 Dahlias 0.8		8 Artichokes 10		9 Artichokes 9.5	
1	Experiment	1		2		3		4		5		6		7		8		9	
2	Type of juice	Pure sugars		Artichokes		Artichokes		Artichokes		Artichokes		Chicory		Dahlias		Artichokes		Artichokes	
3	Temperature of reaction (° C)	0.0		1.0		1.5		3.0		0.5		3.0		0.8		10		9.5	
4	Weight of products, grams:																		
5	Reaction mixture	1,811		1,554		1,715		1,735		1,448		1,644		1,549		1,566		1,838	
6	Waste water	1,199		934		1,121		1,170		849		993		770		1,050		1,234	
7	Wash	724		731		676		705		795		702		675		589		605	
8	Levulate cake	426		444		349		382		406		462		592		394		387	
9	Wash water	(538)		(555)		433		(522)		600		(513)		488		472		400	
10	Product balances:	%	g	%	g	%	g	%	g	%	g	%	g	%	g	%	g	%	g
11	Levulose:																		
12	Reaction mixture	5.38	97.4	8.12	126.1	6.20	106.3	5.88	102.0	5.60	81.2	7.04	115.7	8.92	138.2	6.01	94.2	5.94	109.1
13	Waste water	.23	2.8	1.04	9.7	.91	10.2	.73	8.5	.58	4.9	.84	8.4	.56	4.3	1.25	13.1	1.17	14.4
14	Wash	.23	1.7	.71	5.2	.65	4.4	.47	3.3	.45	3.6	.69	4.8	.49	3.3	.86	5.1	.85	5.1
15	Levulate cake	21.84	93.0	24.10	107.0	26.58	92.8	23.5	89.6	17.71	72.0	21.90	101.2	22.02	130.4	19.29	76.0	22.80	88.2
16	Total recovered		97.5		121.9		107.4		101.4		80.5		114.4		138.0		94.2		107.7
17	Total reducing sugar:																		
18	Reaction mixture	6.55	118.6	10.42	161.9	6.82	117.0	6.80	118.0	6.40	92.8	8.02	131.8	9.55	147.9	8.29	129.9	6.92	127.1
19	Waste water	1.56	18.7	3.70	34.6	2.02	22.7	1.67	19.5	1.52	12.9	1.88	18.7	1.20	9.3	3.55	37.3	2.23	27.5
20	Wash	1.10	7.9	2.19	16.0	1.32	8.9	1.08	7.6	1.00	7.9	1.39	9.8	1.02	6.9	2.34	13.8	1.77	10.7
21	Levulate cake	21.84	93.0	24.10	107.0	26.58	92.8	23.5	89.6	17.71	72.0	21.90	101.2	22.02	130.4	19.29	76.0	22.80	88.2
22	Total recovered		119.6		157.6		124.4		116.7		92.8		129.7		146.6		127.1		126.4
23	Total CaO:																		
24	Reaction mixture	2.53	45.7	3.45	53.6	3.66	62.9	2.71	47.1	3.71	53.7	3.23	53.0	4.00	62.0	3.37	52.7	3.51	64.6
25	Waste water	.37	4.4	1.01	9.4	.69	7.7	.73	8.5	.58	4.9	.67	6.6	.62	4.7	1.01	10.6	.68	8.4
26	Wash	.30	2.2	.62	4.5	.47	3.2	.49	3.5	.40	3.2	.51	3.6	.51	3.5	.71	4.2	.51	3.1
27	Levulate cake	9.08	38.7	8.70	38.6	14.75	51.5	9.00	34.4	11.40	46.3	9.00	41.6	9.06	53.6	10.1	39.8	13.0	50.5
28	Total recovered		45.3		52.5		62.4		46.4		54.4		51.8		61.8		54.6		62.0
29	Ratio, levulose to total reducing sugar:																		
30	Reaction mixture	0.822		0.779		0.910		0.864		0.874		0.878		0.935		0.725		0.858	
31	Waste water	.150		.281		.448		.439		.384		.448		.424		.352		.522	
32	Wash	.209		.325		.494		.439		.450		.493		.487		.369		.48	
33	Yield of levulose, percent	95.5		84.8		87.3		87.8		88.7		87.5		94.3		80.7		80.7	
34	CaO required (theoretical), grams	35.5		47.2		43.4		39.9		30.5		41.7		48.7		38.4		38.9	
35	CaO used, grams	45.7		53.6		62.9		47.1		53.7		53.0		62.0		52.7		64.6	
36	Ratio, CaO to reducing sugar in waste water	0.235		0.272		0.339		0.438		0.383		0.356		0.511		0.285		0.304	

scale experiments they probably represent closely those which may be
expected in a levulose plant of any magnitude.

The percentage of each constituent multiplied by the total weight
of each product (given in lines 5 to 8) yielded the weights of the
respective constituents shown in the right of the subdivided columns.
The sums of the weights of each recovered in the waste water, wash,
and cake are shown under the captions "Total recovered" (lines
16, 22, and 28) and may be compared with the corresponding weights
taken in the reaction mixture (lines 12, 18, and 24). In most instances
the balance is satisfactory.

Experiment 1 shows the product analyses of a mixture of pure
levulose and pure dextrose taken in a ratio of levulose to total sugar
of 82.2 percent (line 30). A yield of 95.5 percent of levulose was
obtained in the washed cake (line 33). Dextrose is here the only
impurity which can exert a solvent effect upon the precipitated cal-
cium levulate. In the waste water which contained 1.56 percent
of total sugar the ratio of levulose to total sugar was 0.150 (line 31),
in agreement with the one which was computed by extrapolating
the values in table 1 to 0° C. The ratio of CaO to reducing sugar
in the waste water (line 36) is in approximate agreement with the
values given in table 1.

The juice used in experiment 2 was obtained from the most com-
monly occurring variety of Jerusalem artichoke in which the ratio of
levulose to total reducing sugar was 0.779 (line 30). The waste
water showed a ratio of 0.281 (line 31), a value far higher than that
obtained from the pure sugars (0.150) in experiment 1. This greater
value of the ratio is to be ascribed to the difructose anhydrides de-
rived from the inulin contained in the juice. Observe further the
ratios (line 31) in the waste waters in experiments 3 to 7, which reach
values nearly 3 times as great as that in experiment 1.

Pure inulin upon hydrolysis by aqueous acids yields a sugar mixture
containing about 92 percent of levulose, 3 percent of dextrose, and
5 percent of difructose anhydrides.[7] The ratio of levulose to total
reducing sugar is about 97 percent. A high ratio of levulose to re-
ducing sugar in the plant juice is therefore an indication of high
inulin content and also an indication of relatively high concentration
of difructose anhydrides after hydrolysis.

The high ratios in the juices taken in the reaction mixtures of experi-
ments 3 to 7 (line 30) indicate that the levulose was derived largely
from inulin and that a considerable concentration of difructose
anhydride must therefore have been present. The ratios of levulose
to total reducing sugar in the waste waters are therefore high. The
juice in the reaction mixture of experiment 2 contained less inulin,
as shown by the low ratio, and therefore less anhydro sugar is formed
upon hydrolysis, and the ratio in the waste water, while nearly twice
as great as that from the pure sugars of experiment 1, is relatively low
compared with the juices of high inulin content.

Fortunately a high inulin content in plant juices is accompanied
by a low dextrose content. Consequently, in spite of the high solvent
effect of the anhydro sugars upon calcium levulate, they actually
cause a smaller loss of levulose than does the dextrose because their
concentration can never become greater than 5 percent of the inulin,

7 Jackson and Goergen, BS J. Research 3, 27 (1929) RP79; Jackson and McDonald, BS J. Research 6,
709 (1931) RP299.

while the dextrose can amount to as much as 25 percent of the total sugar in juices of low inulin content.

The theoretical weight of lime (line 34) was calculated on the assumption that the waste water and wash were saturated and that the levulose in the cake was combined with 1 molecule of CaO. The total theoretical weight is therefore the sum of the weights in the waste water and wash and 31.1 percent of the weight of levulose in the cake. These weights are shown in table 3, together with the weights actually used (lines 34 and 35). It is probable that in many of the experiments the excess of lime used was unnecessarily great.

The ratio of CaO to reducing sugar in the waste water in experiment 1 is 0.235, the solvent effect being produced by dextrose and levulose solely. In the plant juices the ratio assumes greatly increased values, showing again the solvent effect of the difructose anhydrides.

The concentration of levulose (line 12) which can be used in the reaction mixture depends in great measure on the care and skill exercised in building up the precipitate. As has already been shown in table 1, the higher concentrations give higher yields of levulose, but the hazard of producing a finely divided precipitate difficult to filter is increased.

In the experiments recorded in table 3 a wide range of concentrations (line 12) was used and no difficulty in filtration was encountered. In general, while the yields are dependent upon several variables, the higher concentrations of levulose in the reaction mixture appear to cause the more favorable recoveries. In experiment 2 the low ratio of levulose to reducing sugar would ordinarily cause a low recovery, but by taking a concentration of levulose of 8.12 percent in the reaction mixture a favorable yield was obtained. The strikingly high yield of 94.3 percent obtained from dahlia juices in experiment 7 is the combined result of every favorable circumstance, namely, low temperature, high ratio of levulose to total reducing sugar, and very high concentration of levulose in the reaction mixture. The wash water used was, moreover, somewhat less than the weight of the cake, but the last drainings of the wash were essentially colorless. That washing was adequate is shown by the fact that the ratio of levulose to total sugar in the wash (line 32) rose above that of the waste water (line 31), which indicated that pure levulate was being dissolved.

In table 1 it appeared that dextrose held in solution in the waste water roughly one-fifth of its weight of levulose at the lower temperature and lower concentration. The waste water in experiment 7 of table 3 contained 5 g of dextrose (i. e., the difference between total reducing sugar and levulose). The dextrose is thus responsible for the retention of but 1 of the 4.3 g of levulose found in the waste water, the remaining 3.3 g being held back by the difructose anhydrides.

In experiment 1, in which the difructose anhydrides were absent, there are 15.9 g of dextrose in the waste water and there should therefore be about 3 g of levulose. The analytical data show 2.8 g.

A weight of wash water approximately equal to the weight of the levulate cake is sufficient for effective washing. In the present series the washing was continued somewhat after the appearance of a colorless filtrate. By the washing operation a small amount of levulate cake was dissolved. This is shown, in general, by a higher ratio of levulose to total sugar in the wash than in the waste water (lines 31

and 32). In another experiment, not further described here, the final portion of the wash filtrate was analyzed and was found to have a ratio of levulose to total sugar of 95 percent.

Experiments 8 and 9 show again the diminished yields resulting from an increased temperature of precipitation.

In recapitulation, the conditions which favor high yields of levulose are low temperature, high ratio of levulose to total sugar in the crude product, high concentration during precipitation of levulate, and careful control of hydrolysis and defecation.

The question of low temperature of precipitation is complicated by its economic aspect. To facilitate computation we have ascertained by approximate calorimetric measurement that in the reaction between 1 g of levulose and an excess of milk of lime there is an evolution of about 70 calories, between dextrose and lime milk about 31 calories. The specific heat of milk of lime containing 22.4 percent of CaO was found to be 0.78. Unfortunately we have no experimental data on the specific heat of the defecated juice containing 15 percent of levulose, but a juice containing 23.5 percent of levulose and 6.6 percent of dextrose had a specific heat of 0.77, which permits a rough estimate that a juice of similar purity diluted to 15 percent of levulose would have a specific heat of about 0.85. Obviously more refined data are desirable.

The ratio of levulose to total sugar is subject to control only by selection of varieties of artichokes which have a high ratio. There are in existence many varieties of artichokes among which considerable differences occur in respect of the composition of the juices. Some are characterized by a high ratio of levulose to total sugar and are more valuable as sources of levulose. All varieties apparently show their highest ratio in the early autumn immediately after the plant has matured. The ratio thereafter gradually diminishes by transformation of polysaccharides which are composed of levulose to substances which yield dextrose upon hydrolysis. If the dahlia and chicory could be grown as inexpensively as the artichoke they would be greatly superior as sources of levulose.

III. PURITIES OF THE DERIVED LEVULOSE SOLUTIONS

The preparation of the calcium levulate described in the preceding paragraphs was carried out with care, and yet upon suspension in water, carbonation, and evaporation, the levulose sirups emerging from the process showed apparent purities of but 91 to 97 percent, as in the original investigation. It became evident that so great an amount of impurity could not have been retained in the levulate cake, for a simple calculation showed that if the cake had been left unwashed a purity of about 94 percent would have resulted. The thorough washing which the levulate cakes actually received should have removed most of the impurity. Careful analysis enabled us to determine the nature of the impurities in the final liquor and led ultimately to their essential elimination. The principal impurities proved to be dextrose, calcium, magnesium, sulphur as sulphates, and aluminum.

Consider first the dextrose and its origin. It is improbable that dextrose was precipitated with calcium levulate since the amount found in the final solution was about the same whether we started with invert sugar of 50-percent dextrose purity or with hydrolyzed

inulin of about 3-percent purity. It was thought possible that dextrose was formed by the Lobry de Bruyn-van Ekenstein rearrangement during carbonation of the levulate cake, since during the early stages of the carbonation the liberated levulose dissolved levulate and produced a high alkalinity. We have shown, however, in a previous article [8] that the rearrangement at low temperatures is extremely slow even at far higher alkalinities than are present during carbonation. A solution normal with respect to hydroxyl ions requires about 6 hours for a 1-percent decomposition of levulose, whereas but 5 to 10 minutes was required for the carbonation. It is, moreover, improbable that the alkalinity became as great as normal.

An experiment was carried out in which a great excess of carbon dioxide was used in order to produce a calcium bicarbonate solution, the levulate cake being added in small portions. At no time was the solution alkaline to phenolphthalein and yet the resulting levulose solution showed but 95-percent purity.

It ultimately proved that the dextrose found in the final liquors was formed by the Lobry de Bruyn-van Ekenstein rearrangement during the evaporation of the carbonated solution. The carbonation of the levulate cake was necessarily carried to a point at which an appreciable amount of calcium bicarbonate was formed. The precipitated calcium carbonate was removed by filtration and the filtrate containing calcium bicarbonate evaporated to release carbon dioxide and precipitate the remaining calcium carbonate. It was at this point that the rearrangement occurred, for when calcium carbonate is produced in this way it imparts a pH of 9.0 to 9.5 to the solution.[9] This alkalinity at 40 to 60° C is sufficient to cause the rearragement.[10]

This particular region according to Mathews and Jackson's data is characterized by the great variation of stability of levulose with relatively small changes of pH and temperature. At 40° C and pH 9, 3.1 hours are required for a 1-percent decomposition of levulose; at 60° C the time is diminished to 10 minutes; at pH 10 and 40° C the time is 30 minutes and at 60° C 1.7 minutes. In view of so great a sensitiveness to small changes in conditions it is now not surprising that in previous preparations great fluctuations in the purities of the levulose sirups occurred.

It is probable that in many instances the degradation of the sugar proceeded in appreciable amount to the formation of acids which accounts for the presence of calcium and magnesium in the final sirups.

The sulphates which contaminated the sirup were derived from the calcium sulphate produced during the defecation of the hydrolyzed juice. It proved to be difficult to induce calcium sulphate to precipitate from these solutions even when its concentration was far above its solubility in pure water and even when seeded with gypsum. In one experiment the defecated juice contained 0.96 percent of calcium sulphate after filtration at 24° C, a saturated solution in pure water containing 0.21 percent. The amount remaining in the defecated juice was practically equivalent to the sulphuric acid used for hydrolysis.

During the precipitation process a small quantity of calcium sulphate precipitated with the levulate, possibly because the great

[8] BS J. Research 11, 632 (1933) RP611.
[9] J.Soc.Chem.Ind. 41, 311 (1923).
[10] Mathews and Jackson, BS J. Research 11, 632 (1933) RP611.

excess of calcium ions increased the ion product beyond the solubility product, or possibly by some form of occlusion. In the most favorable instances 0.08 percent of SO_3, based upon dry substance, was found in the final sirups. In one of the less favorable instances 0.2 percent of SO_3 was found present. In the later experiments a quantity of barium carbonate or barium hydroxide slightly in excess of the calculated requirement was added previous to the carbonation of the levulate cake. This method is capable of removing sulphates effectively, although in only one instance did we add the barium in sufficient quantity.

The most persistent impurities which have not thus far yielded to treatment proved to be magnesium and aluminum, together with small quantities of iron and silica. The magnesium was derived from the lime and remained insoluble as magnesium hydroxide during the precipitation and filtration of calcium levulate. During the carbonation step it was converted to carbonate, which had an appreciable solubility (10.6 mg in 100 ml of water) and thus remained in the final solution. It is conceivable that magnesium carbonate contributes as much as or possibly more than calcium carbonate to the alkalinity of the carbonated solution. We propose to experiment further with magnesia-free lime. The aluminum and iron cycle has not been investigated.

Since dextrose formed by rearrangement of levulose was the predominating impurity in the final sirups, it was imperative to remove calcium bicarbonate by some other means than by volatilization of carbon dioxide in order that an alkaline reaction should be avoided during evaporation. In the procedure finally adopted this was done immediately upon completion of the carbonation, which was carried out in cold solution. In practice the carbonation was continued to a point where the solution was acid to phenolphthalein, an excess of carbon dioxide being necessary to decompose the more resistant particles of levulate and to combine with any free lime which might have been present. A very thin lime milk or calcium levulate suspension was then added to transform calcium bicarbonate to carbonate. At first the attempt was made to control this addition by pH measurements, but this method in so thick a suspension proved impracticable. The most delicate control was obtained by the method of conductometric measurement, since the only conducting substance present in considerable quantity was calcium bicarbonate. The conductivity cell which was of a rugged type permitting free circulation of the solution was inserted directly in the carbonated sludge and the lime milk added slowly until the mixture showed a minimum conductivity. A small portion of the original suspension was held in reserve in order that the end point could be readjusted when it was once passed. The calcium was now in the carbonate stage and had its minimum solubility. Similarly the magnesium was precipitated to the extent of the solubility of magnesium carbonate. Incidentally this method of removing bicarbonate corrects in some measure any errors of defecation, for the solution is alkaline to phenolphthalein, a higher pH than occurs in the usual defecated juice. At this point the solution was still cold and no appreciable decomposition occurred.

The sludge adjusted to minimum conductivity was filtered while still cold. The slightly alkaline filtrate was then treated with dilute

oxalic acid to exactly quantitative precipitation of calcium. Here again the acidification was precisely controlled by the conductivity method, the solution being adjusted to a maximum resistance. The adjustment can be made deliberately and results in a quantitative elimination of calcium. Since only enough oxalic acid is required to neutralize a saturated solution of calcium and magnesium carbonates the amount used is small and by rough calculation its cost is but a few hundredths of a cent per pound of levulose. Probably in a large-scale operation the calcium oxalate could be accumulated and the oxalic acid regenerated.

There is of course objection to the use of oxalic acid and it is our purpose to investigate alternative methods in continuation of the work.

At the point of minimum conductivity the pH was approximately 4.7, and rose to about 5.0 upon evaporation to about 20 Brix. A pH measurement was, however, found unsuitable for the control of the oxalic-acid addition since with the elimination of inorganic salts the solution was but weakly buffered and minute quantities of impurities altered the pH profoundly.

We have shown in a previous article [11] that levulose in water solution has a maximum stability at pH 3.3   At pH 5 it is still sufficiently stable to withstand the conditions to which it is subjected during evaporation and crystallization. It would be a simple matter, if necessary, to reduce the pH to 4, at which it is 2½ times as stable as at 5.

After removal of calcium oxalate by filtration the solutions were evaporated to some density at which it is advantageous to refilter, since usually there occurred a further separation of inorganic salts during evaporation. At the time of refiltration about 2 percent of Darco, based on dry substance, was added, resulting always in a considerable improvement in the apparent purity, which was in some instances increased a whole unit. The carbon proved particularly effective in color removal and the final sirups were practically water white even when evaporated to above 80 Brix.

The sirups were subjected to a careful analysis. By application of the Mathews formula [12] it was determined that the liquors were free from dextrose, the results approximating 100-percent ratio of levulose to total sugar within the limits of analytical precision. Dry substance was determined by means of the immersion refractometer and levulose by direct polarization. Ash was determined by incineration below 600° C without the addition of sulphuric acid. Sulphates were determined in the original sirup.

In table 4 are shown the purities which can be obtained by the modification of the process of recovery of levulose from the levulate precipitate. Although some change of procedure was made in each experiment, all embodied the principle of avoiding alkalinity during evaporation. It is evident that the residual impurity consists mainly of inorganic salts, among which magnesium and aluminum predominate.

In the first 2 experiments a sample of lime practically free from magnesia was used. The high quality of the lime is reflected in the high purity and low ash content of the sirup. In the remaining

[11] BS J. Research 11, 619 (1933) RP611.
[12] BS J. Research 8, 433 (1932).

TABLE 4.—*Purities of levulose solutions derived from the calcium levulate process*

Experiment	Raw juice[a]	Purity of filtrate	Purity after addition of 2% of Darco	Ratio, levulose to total sugar	Ash on dry substance	Analysis of ash				
						CaO	MgO	R₂O₃[b]	SO₃	Undetermined
		%	%	%	%	%	%	%	%	%
1	1		99.10		0.25					
2	1		99.21		.28					
3	2	98.70	99.08		.42					
4	2	97.99	99.02		.51					
5[c]			99.27	100.4	.46	6.0	35.1	15.1	17.5	26.3
6	2	97.50	98.26	100.0	.71					
7	2	98.96	99.22	99.4	.51	0	39.0	28.4	6.8	25.8
8	2	98.53	98.93		.53	0	40.1	14.8	1.5	43.7

[a] Raw juice from artichokes.   Number 1 had a ratio of levulose to total sugar of 74.2 percent; number 2, 77.0 percent.
[b] Aluminum and iron oxides and substance insoluble in HCl.
[c] Solutions 3 and 4 combined and treated again with 2 percent of Darco.

experiments lime containing about 2 percent of magnesia was used. In experiment 6 the bicarbonate was destroyed by evaporation of carbon dioxide, the solution being kept acid by constant additions of oxalic acid.   This procedure allowed most of the magnesium to remain in the sirup, whereas in the remaining experiments much of the magnesium was precipitated as carbonate.

The data in table 4 show that from crude plant juices, which usually have a levulose purity of about 65 percent, final sirups having a purity in excess of 99 percent can be recovered.   Moreover, since the residual impurity consists of relatively insoluble inorganic salts, it may prove in further experimentation that they can at a suitable dilution be partially removed by filtration, in which case the purities of the mother liquors from crystallization will remain high.   In any case it is evident that sirups of such high purity will yield a considerable amount of crystalline sugar before the final molasses requires recirculation.

IV. PURITY TABLES

The method of determination of the purity of levulose sirups depends upon the degree of purity of the sample.   If the substance is uncontaminated by dextrose a direct polarization yields a correct measure of the levulose concentration.   If dextrose is also present and is the only optically active or reducing impurity the ratio of levulose to total sugar can be determined by means of the Mathews formula.[13]   If other optically active or reducing impurities than dextrose occur in the sample, levulose must be determined by Nyns's selective reduction method.[14]   Dry substance is determined most conveniently by density or refractive-index measurement.

For the determination of purity of the solutions obtained by the method described in the present article a determination of the direct polarization and dry substance is generally sufficient.

Since the labor of calculating purities from these observations is considerable, we have sought to facilitate the computation by the preparation of purity tables which permit the transformation of observations to purities in a single operation.   The necessary factors

[13] BS J. Research **8**, 433 and 442 (1932) RP426.
[14] BS J. Research **8**, 422 (1932) RP426.

are given in tables 5 to 7.   In table 8 are given some physical prop-
erties of levulose solutions and their temperature coefficients.   These
data, compiled and recalculated from observations published in a
previous article,[15] are serviceable for making small adjustments for
temperature and for determining concentrations of levulose.

The sample, if necessary, is diluted with sufficient water to yield
a polarization in a 2-dm tube within the scale of the saccharimeter.
The density or the refractive index is then determined.   For deter-
mination of the density all weights must be corrected to a vacuum
and the volume measurement made at or corrected to exactly 20 or
25° C.   A column of density-temperature coefficients is given in table
8 to facilitate making the usually small corrections to either of the
standard temperatures.

The refractive index is determined either with an instrument of
the Abbe type or with an immersion refractometer.   Table 8 gives
factors which are applicable only to the arbitrary scale originally
proposed by Pulfrich,[16] according to which 14.5=1.33300, 50.0=
1.34650, and 100.0=1.36464.   If other arbitrary scales are used the
readings must either be corrected to Pulfrich's scale or transformed
to refractive indices and the latter applied in table 7.   Temperature
coefficients are given in table 8 to simplify corrections to the standard
temperatures.

The direct polarization is made preferably at exactly 20 or 25° C.
If made at other temperatures it must be corrected to one of these
temperatures.   A column of polarization-temperature coefficients in
table 8 includes the change in rotation and the change in volume with
change of temperature.   If the temperature of the quartz wedges
differs from that of the solution a correction should be applied to
bring the temperature of the quartz to the temperature of the solu-
tion before the coefficients in table 8 are applied.   The correction for

TABLE 5.—*Factors for obtaining purities from direct polarization and density*

[Polarization in 2-dm column and density must be observed at or corrected to the same temperature, either
20 or 25° C. Opposite the corrected density find the factor by interpolation. Substitute the factor and
the corrected polarization and density in eq 1 or 2]

$$\frac{\text{Factor} \times \text{polarization at } 20° \text{ C}}{100(D_4^{20}-0.99823)}=\text{percentage purity.} \tag{1}$$

$$\frac{\text{Factor} \times \text{polarization at } 25° \text{ C}}{100(D_4^{25}-0.99708)}=\text{percentage purity.} \tag{2}$$

$D_{4°}^{20 \text{ or } 25}$	Factor		$D_{4°}^{20 \text{ or } 25°}$	Factor	
	20° C	25° C		20° C	25° C
1.000	7.335	7.516	1.040	7.214	7.396
1.005	7.320	7.501	1.045	7.198	7.380
1.010	7.305	7.487	1.050	7.182	7.363
1.015	7.290	7.472	1.055	7.165	7.345
1.020	7.275	7.458	1.060	7.148	7.327
1.025	7.260	7.444	1.065	7.130	7.309
1.030	7.245	7.429	1.070	7.112	7.290
1.035	7.230	7.412	1.075	7.093	7.271

[15] BS J. Research 8, 403 (1932) RP426.
[16] Z. angew. Chem. p. 1186 (1899).

quartz is 0.000144° S per degree temperature per saccharimeter degree. If the temperature of the wedges is higher than that of the solution the correction is to be added; that is, the negative rotation of the solution is to be increased to a higher negative value. No correction is made for concentration of sugar.

Having determined the polarization and either density or refractive index, substitute the corrected observations in the respective equations in tables 5 to 7 for the determination of purity.

TABLE 6.—*Factors for obtaining purities from direct polarization and refractive index*

Polarization in 2-dm column and refractive index must be observed at or corrected to the same temperature, either 20 or 25° C. Opposite the corrected refractive index find the factor by interpolation. Substitute the factor and the corrected polarization and refractive index in eq 3 or 4]

$$\frac{\text{Factor} \times \text{polarization at } 20° \text{ C}}{100 (n_D^{20} - 1.33300)} = \text{percentage purity.} \tag{3}$$

$$\frac{\text{Factor} \times \text{polarization at } 25° \text{ C}}{100 (n_D^{25} - 1.33250)} = \text{percentage purity.} \tag{4}$$

n_D	Factor		n_D	Factor	
	20° C	25° C		20° C	25° C
1.333			1.347	2.629	2.704
1.334	2.664	2.760	1.348	2.626	2.699
1.335	2.661	2.754	1.349	2.622	2.695
1.336	2.659	2.749	1.350	2.619	2.691
1.337	2.656	2.742	1.351	2.615	2.687
1.338	2.654	2.737	1.352	2.611	2.684
1.339	2.651	2.732	1.353	2.608	2.680
1.340	2.649	2.728	1.354	2.604	2.676
1.341	2.646	2.724	1.355	2.600	2.671
1.342	2.642	2.720	1.356	2.596	2.667
1.343	2.640	2.717	1.357	2.592	2.664
1.344	2.636	2.713	1.358	2.589	2.660
1.345	2.634	2.711	1.359	2.585	2.657
1.346	2.632	2.708	1.360	2.581	2.654

TABLE 7.—*Factors for obtaining purities from direct polarization and immersion refractometer readings*

[The factors are applicable only to readings made on refractometers whose arbitrary scale corresponds to Pulfrich's definition; thus, 14.50=1.33300, 50.0=1.34650, 100=1.36464. Polarization in 2-dm column and refractive index must be made at or corrected to the same temperature, either 20 or 25° C. Opposite the corrected immersion reading find by interpolation the factor. Substitute the factor and the corrected polarization and immersion reading in eq 5 or 6]

$$\frac{\text{Factor} \times \text{polarization at } 20° \text{ C}}{\text{Immersion reading at } 20° \text{ C} - 14.50} = \text{percentage purity.} \tag{5}$$

$$\frac{\text{Factor} \times \text{polarization at } 25° \text{ C}}{\text{Immersion reading at } 25° \text{ C} - 13.25} = \text{percentage purity.} \tag{6}$$

Immersion refractometer reading at 20 or 25° C	Factor		Immersion refractometer reading at 20 or 25° C	Factor	
	20° C	25° C		20° C	25° C
15	68.75	70.60	45	69.11	70.99
20	68.83	70.70	50	69.15	71.00
25	68.90	70.79	60	69.20	71.02
30	68.96	70.87	70	69.23	71.03
35	69.02	70.93	80	69.25	71.04
40	69.07	70.97	90	69.25	71.05

TABLE 8.—*Physical properties of levulose solutions*

Concentration (g/100g of solution)		Levulose in 100 ml (vacuum) at—		Levulose in 100 ml (air, brass weights) at—		$\frac{\Delta(g/100\ ml)}{\Delta T}$ $\times 10^{-5}$ $\times(-1)$	$D\,{}^{20}_{4}$ (vacuum)	$D\,{}^{25}_{4}$ (vacuum)	Weight of 1 ml (air, brass weights) at—		$-\frac{\Delta D}{\Delta T}$ $\times 10^{-6}$	$n\,{}^{20}_{D}$	$n\,{}^{25}_{D}$	$\frac{\Delta n}{\Delta T}$ $\times 10^{-6}$ $\times(-1)$	Immersion readings at [1]		$\frac{\Delta Imm.}{\Delta T}$	Polarization [2] at—		$-\frac{\Delta Pol.\,[3]}{\Delta T}$ $\times(-1)$
True (in vacuum)	Apparent (in air vs. brass weight)	20° C	25° C	20° C	25° C				20° C	25° C					20° C	25° C		20° C	25° C	
%	%	g	g	g	g				g	g								°S	°S	
0	0	0	0	0	0	0	0.99823	0.99708	0.99717	0.99602	231	1.33300	1.33252	96	14.50	13.25	0.250	0	0	0
1	1.0004	1.0021	1.0010	1.0015	1.0003	24	1.00214	1.00095	1.00108	0.99989	238	1.33442	1.33393	98	18.18	16.90	.256	5.33	5.15	0.036
2	2.0009	2.0121	2.0097	2.0109	2.0085	49	1.00607	1.00484	1.00501	1.00378	245	1.33585	1.33535	100	21.87	20.58	.262	10.72	10.35	.073
3	3.0013	3.0301	3.0263	3.0282	3.0245	76	1.01003	1.00877	1.00897	1.00771	252	1.33729	1.33678	102	25.63	24.29	.268	16.15	15.60	.110
4	4.0017	4.0561	4.0509	4.0536	4.0484	104	1.01402	1.01272	1.01296	1.01166	259	1.33874	1.33822	104	29.43	28.06	.274	21.65	20.91	.147
5	5.0021	5.0901	5.0835	5.0870	5.0804	133	1.01803	1.01670	1.01698	1.01565	266	1.34020	1.33967	106	33.27	31.88	.280	27.19	26.27	.184
6	6.0025	6.1324	6.1243	6.1287	6.1205	164	1.02207	1.02071	1.02102	1.01966	273	1.34167	1.34113	108	37.14	35.72	.286	32.80	31.69	.222
7	7.0029	7.1830	7.1733	7.1786	7.1689	196	1.02614	1.02475	1.02509	1.02370	280	1.34315	1.34260	110	41.06	39.62	.292	38.46	37.16	.260
8	8.0033	8.2419	8.2305	8.2369	8.2255	230	1.03024	1.02881	1.02919	1.02776	287	1.34464	1.34408	112	45.04	43.54	.298	44.19	42.69	.299
9	9.0037	9.3093	9.2961	9.3037	9.2905	265	1.03437	1.03290	1.03332	1.03185	294	1.34615	1.34558	115	49.06	47.54	.304	49.97	48.28	.338
10	10.0040	10.3853	10.3702	10.3790	10.3639	301	1.03853	1.03702	1.03748	1.03597	301	1.34766	1.34708	117	53.13	51.56	.312	55.81	53.93	.377
11	11.0044	11.4698	11.4530	11.4628	11.4460	339	1.04271	1.04118	1.04166	1.04013	308	1.34918	1.34858	119	57.21	55.59	.320	61.72	59.64	.417
12	12.0047	12.5630	12.5442	12.5554	12.5366	378	1.04692	1.04535	1.04587	1.04430	315	1.35071	1.35010	121	61.34	59.70	.328	67.70	65.41	.457
13	13.0051	13.6651	13.6441	13.6568	13.6359	420	1.05116	1.04955	1.05011	1.04850	323	1.35225	1.35163	123	65.53	63.83	.336	73.74	71.25	.498
14	14.0054	14.7760	14.7529	14.7670	14.7439	462	1.05543	1.05378	1.05438	1.05273	330	1.35380	1.35317	125	69.77	68.02	.344	79.84	77.15	.539
15	15.0057	15.8958	15.8706	15.8861	15.8610	506	1.05972	1.05804	1.05867	1.05699	337	1.35535	1.35471	127	74.05	72.27	.352	86.02	83.11	.581
16	16.0061	17.0248	16.9973	17.0144	16.9869	552	1.06405	1.06233	1.06300	1.06128	345	1.35692	1.35627	129	78.38	76.58	.360	92.26	89.15	.623
17	17.0064	18.1628	18.1329	18.1518	18.1219	598	1.06840	1.06664	1.06735	1.06559	352	1.35850	1.35784	132	82.78	80.95	.368	98.58	95.25	.667
18	18.0067	19.3100	19.2776	19.2983	19.2659	648	1.07278	1.07098	1.07173	1.06993	360	1.36009	1.35943	135	87.21	85.31	.376	104.97	101.42	.711
19	19.0070	20.4666	20.4316	20.4542	20.4192	697	1.07719	1.07535	1.07614	1.07430	367	1.36170	1.36103	137	91.67	89.74	.385	------	------	------
20	20.0073	21.6324	21.5950	21.6193	21.5819	750	1.08162	1.07975	1.08057	1.07871	375	1.36333	1.36263	139	96.17	94.20	.394	------	------	------

[1] Referable solely to the scale of arbitrary units proposed by Pulfrich, Z. angew. Chem. p. 1,186 (1899). According to this scale, 14.5=1.33300; 50.0=1.34650; and 100.0=1.36464.

[2] Polarization in 2-dm column in saccharimeter whose scale conforms to the specification that the normal quartz plate rotates 40.690° for λ=5,461 A, and 34.620° for λ=5,892.5 A at 20° C.

[3] These coefficients include the change of volume as well as change of rotatory power. They can be applied directly to give the rotation of the solution at either of the standard temperatures.

Washington, July 12, 1925.