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EXTRACTION OF GRAINS AND CATTLE FOODS FOR THE DETERMINATION OF SUGARS: A COMPARISON OF THE ALCOHOL AND THE SODIUM CARBONATE DIGESTIONS.

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INTRODUCTION.

There has been some discussion among chemists as to methods of determining sugars in agricultural products, such as grains, plants, both dried and fresh, and cattle foods. Figures obtained by different chemists on the same sample varied many times by 1 per cent or more. This wide variation in results was also noted when the same chemist used different methods or made a slight modification of his own in a printed method. The difficulty experienced was not so much in the actual determination of the sugar as in the solvent and the method of procedure used to obtain an extract on which to work. Again, sometimes the results obtained on one day could not be duplicated on the same sample at a later date. Often this may be attributed to lack of homogeneity of the sample, a fault remedied by better preparation of the material, especially by finer grinding. But the chief difficulty appears to lie in the changes due to enzymic action during or after extraction and also somewhat to the solvent and the clarification agents used. In this class of material, sugars are usually present in small quantities and the other carbohydrates, such as starch, pentosans, gums, etc., in much larger amounts. It is because of the latter substances that the usual water methods for sugar-beet analysis can not be successfully used.

LITERATURE ON THE SUBJECT.

The literature contains but few definite methods for the determination of sugars in grains, although alcohol is often spoken of as the solvent used for extracting materials, especially when sugars are to be obtained. Other references are found to the determination of

sugars in the water extract of the material. In the early methods¹ of the Association of Official Agricultural Chemists, the procedure was to "stir 3 grams of the sample in a beaker with 50 cc of water for an hour," filter, and make up to volume. This procedure gave varying results, depending upon the temperature of the water during extraction and the fineness of the material. Later this procedure was modified by using ice water for extraction and submerging the beaker during extraction in a bath of broken ice.² In a majority of cases this does not wholly accomplish its purpose, namely, to stop enzymic action and thereby insure concordant results. It is a rather cumbersome procedure, and, while enzymes may be inhibited during extraction, they become active again when the filtration is carried on at room temperature. However, many now use this method of procedure for preparing the extract for sugar determination. The official methods offer as an alternate for the water method an extraction with 40 or 50 per cent alcohol, but no definite procedure is outlined.

O'Sullivan, as early as 1886, was using alcohol to extract barley for the determination of the quantity and kind of sugars present. He states this to be the usual method of determining sugars at that time. Stone,³ in 1897, published a method for "The Quantitative Determination of Carbohydrates in Foodstuffs," in which he extracted successively with boiling alcohol, cold water, diastase or malt infusion, dilute hydrochloric acid, and finally 1.25 per cent sodium hydroxid. The alcohol treatment was for the removal of the true sugars, the cold water for the removal of dextrin and soluble forms of starch. Browne⁴ published results obtained on a distillery waste by this method, but beyond this little mention of it could be found.

M. N. Straughn, when working with C. G. Church on the sugar content of dried sweet corn at the Maryland Experiment Station in 1902, tried many modifications of the water extraction and alcohol extraction methods. In this product sugars were present in small quantities together with a large percentage of starch, and the enzymes were active, as the drying had been carried on at a low temperature. On extracting this material with cold water great difficulties were experienced in obtaining clear extracts and a rapid filtration. It was soon found that the percentage of total sugars obtained by this method increased with the length of time of extraction, or, if the time of extraction was fixed, the results varied with the time consumed in filtering. This increase was no doubt due to enzymic action. Extraction with hot water could not be practiced, as the material gelatinized, some becoming soluble, and filtration was almost impossible.

U. S. Dept. Agr., Bureau of Chemistry Bul. 46, p. 24.

² U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised, p. 57.

J. Amer. Chem. Soc., 1897, **29**: 183.

J. Amer. Chem. Soc., 1901, **23**: 229.

Alcoholic digestion was then tried. By this method duplicate results were obtained on different days, and there was an agreement of results whether cold or hot solvents were used, although with cold extraction great difficulty was experienced at times in filtering, but even under these circumstances the results of the sugar determination in the extract, provided evaporation was guarded against, were practically the same. In a study made on the "Influence of Environment on the Composition of Sweet Corn,"¹ the alcohol digestion method was used.

The sugar laboratory in 1905 conducted a long series of experiments on the various methods of extraction and the solvents to be used for dissolving the sugars from grain. The results confirmed those of Church and Straughn on the whole. It was found that 50 per cent alcohol was the best solvent and that the extraction should be carried on hot. At this strength it was noted that all enzymic action seemed to be stopped and the extracts were easily filtered. The method was tried on many dried plants, grains, and numerous other materials sent to this laboratory for examination. Slight modifications have been made in the procedure of the method from time to time, and its final statement is given in the following section.

STATEMENT OF ALCOHOL DIGESTION METHOD.

Place 12 grams of material in a 300-cc graduated flask,² add 150 cc of 50 per cent alcohol by volume (carefully neutralized), mix thoroughly, and boil on a steam bath for one hour, using a small funnel in the neck of the flask to condense the vapor. Then cool. If desired, allow it to stand overnight. Make up to volume with 95 per cent alcohol (neutral in reaction), mix thoroughly, allow to settle, transfer 200 cc to a beaker with a pipette, and evaporate on steam bath to a volume of from 20 to 30 cc. The presence of a trace of alcohol is not harmful. Do *not* evaporate the solution to dryness. (By evaporating the 200 cc portion in a short-necked, balloon-shaped distilling flask, connected with a condenser, 75 to 80 per cent of alcohol can be recovered before the material in the flask foams violently. The short neck—1 inch—of these flasks makes it possible to remove the residue easily. The 100 cc remaining in the digestion flask may be strained through a cotton bag and the alcohol recovered from the liquid as just described. This is easily accomplished and results in a marked saving when a large number of samples are run.) Transfer the contents of the beaker or flask, as the case may be, to a 100-cc graduated flask, washing thoroughly with water. Add enough of a saturated solution of neutral lead acetate to produce a flocculent pre-

¹ U. S. Dept. Agr., Bureau of Chemistry Bul. 127.

² If the material is acid in reaction, it would be well to add from 1 to 3 grams of precipitated calcium carbonate to neutralize the acidity.

precipitate, and allow to stand 15 minutes. At this point the solution may safely stand overnight if desired. Make up to the mark with distilled water and pass through a folded filter, carefully saving all of the filtrate, to which add enough anhydrous sodium carbonate to precipitate all the lead, allow to stand 15 minutes, and pour onto an ashless filter. Over 75 cc of filtrate should be obtained. Test the filtrate for lead with a small quantity of dry sodium carbonate, and if any precipitation occurs add more anhydrous sodium carbonate and refilter. Use 25 cc of this clear filtrate together with 25 cc of water for the determination of reducing sugars by the method of Munson and Walker.¹ In a 100-cc graduated flask, place 50 cc of the same filtrate, add a small piece of litmus paper, and neutralize with acetic acid; then add 5 cc of concentrated hydrochloric acid, and let stand overnight for inversion. (Standing 48 hours does not apparently affect the results.) Then pour the inverted solution into a 400-cc beaker and neutralize with anhydrous sodium carbonate; return it to the 100-cc flask and make up to the mark. Filter, if necessary, and use 50 cc for the determination of total sugars as invert by the method of Munson and Walker.²

The amount of cuprous oxid or copper obtained (see page 7) in either the reducing or the total sugar determination represents the sugar contained in 2 grams of the material. Therefore the weights of the invert sugar when divided by 2 and multiplied by 100 give the respective per cents of sugar as invert. Subtract the per cent of reducing sugars before inversion from the per cent of total sugar after inversion, both calculated as invert, and the difference multiplied by 0.95 gives the per cent of sucrose (see page 13 for expression of results). Since the insoluble material of the grain or cattle food occupies some space in the flask as originally made up, it is necessary to correct for this volume. Results of a large number of determinations on various materials have shown the average volume of 12 grams of material to be 9 cc; therefore the correction factor for 12 grams in 300 cc is 0.97, and the percentage figures for reducing sugar and sucrose are to be multiplied by this factor to obtain the true amounts.

STATEMENT OF SODIUM CARBONATE DIGESTION METHOD.

Lately the cold-water extraction method has been improved. Realizing that the trouble experienced with this method of extraction was largely due to the activity of enzymes, it was suggested that a small percentage of sodium carbonate be added to the water used for extraction to inhibit their action.³ Based upon this suggestion,

¹ U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised, p. 241.

² Ibid.

³ J. Ind. Eng. Chem., 1909, 1: 299.

a method was devised which in the case of certain grains gave figures that checked with those obtained by the alcohol digestion. The procedure is as follows:

Place 8 grams¹ of the finely ground grain in a 250-cc flask and add 190 cc of a 0.2 per cent solution of sodium carbonate. Allow to stand at room temperature for two and one-half hours, shaking intermittently. At the expiration of the time add 10 cc of a hot saturated potassium alum solution; shake and filter. Use 25 cc of the solution, the equivalent of 1 gram of material, for reduction by Allihn's method,² calculating the cuprous oxid obtained (see page 7) to dextrose by Allihn's table. To 50 cc of the solution in a 50 to 55 cc flask, add 5 cc of concentrated hydrochloric acid and allow to stand overnight for inversion. Then neutralize the whole solution with sodium carbonate, make up to 100 cc, and in 25 cc of this (equivalent to half a gram of material) again determine the total reducing sugar by Allihn's method. Sucrose (see page 13) is obtained by multiplying the percentages of dextrose before and after inversion by the factor 1.044,³ then subtracting and multiplying the figure thus obtained by 0.95. No correction of the percentages obtained is necessary for the volume of the material, but when working with very wet material the results should be corrected for the dilution caused by the moisture content of the sample.

COMPARISON OF THE TWO METHODS.

The sodium carbonate digestion method having given good results on grains, the authors decided to test it in comparison with the alcohol method on various classes of material and to determine its limitations or the chance of error from differences in procedure. A number of representative samples were selected and comparative determinations made. The results obtained on samples of corn, wheat, and milo are given in the following table. These, and all subsequent results, are expressed as invert sugar.

Results on feeding stuffs by both methods.

Serial No.	Substance.	Alcohol digestion method.		Sodium carbonate digestion method.	
		Reducing sugars.	Total sugars.	Reducing sugars.	Total sugars.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
7893	Corn.....	0.07	1.75	0.21	1.89
7917	Wheat.....	.07	2.35	.20	3.00
7916	Milo.....	.15	1.38	.25	1.43

¹ Where only total sugars after inversion are to be determined and the quantity of total sugars is small 8.8 grams are used and extracted in the usual way. Fifty cc of the solution and 5 cc of acid are inverted overnight. The 55 cc are neutralized with dry sodium carbonate and 25 cc used for reduction. The figures obtained for dextrose represent the amount from 1 gram of material.

² U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised, p. 49.

³ U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised, p. 53.

These figures show that the two methods give results that are fairly comparable. In the case of the wheat, however, the sodium carbonate method gave results for total sugars that are a little higher than those obtained by the alcohol method. This no doubt is due to the fact that trouble was experienced in filtering the extract quickly and clearly after adding the alum, hence allowing the enzymes to become active again, a point which will be considered later in the discussion.

Samples of mixed cattle foods were then examined by the two methods, with the following results:

Examination of mixed cattle foods by the two methods.

Serial No.	Substance.	Alcohol digestion method.		Sodium carbonate digestion method.	
		Reducing sugars.	Total sugars.	Reducing sugars.	Total sugars.
7856-A	Corn, cotton-seed meal, and alfalfa.....	<i>Per cent.</i> 0.00	<i>Per cent.</i> 2.99	<i>Per cent.</i> 0.42	<i>Per cent.</i> 3.30
7935	Corn, alfalfa ¹	.09	.26	.43	.26

¹ This sample had undergone fermentation.

It is to be noted that the sodium carbonate digestion method gives a perceptible quantity of reducing sugars, determined from the weight of the precipitated cuprous oxid. On examination of the cuprous oxid precipitate obtained in the sodium carbonate digestion method it was found that considerable organic matter was occluded in the precipitate, and also some mineral matter. This was traced back to the alum clarification, as alum does not clarify as completely as lead acetate. Using neutral lead acetate instead of alum for clarification in a second set of determinations on this material, the reducing sugar of the first sample came down to 0.00 and of the second sample to 0.08 per cent. With the lead clarification it is seen that the results compare more closely.

With other materials, such as molasses feeds and plants, high in nitrogenous bodies, when alum was used as a clarifying agent, it was noted that the precipitated cuprous oxid in the direct reducing-sugar determination was badly contaminated, a greenish flocculent mass occurring quite often. When neutral lead acetate was used no indication of this greenish precipitate was noted in the determination of reducing sugars in these samples. This shows alum to be a poor clarifying agent for sugar determinations by this method and indicates that neutral lead acetate should be used for this purpose. In no case, however, should lead subacetate be used as a clarifying agent, as its power of precipitating reducing sugars is well known.¹

¹ Intern. Sugar Journal, 1908, 10: 602.

As has been suggested, errors are likely to be introduced in the cuprous oxid weighing method by the presence of mineral salts and organic matter. Fehling solution being strongly alkaline will cause in some cases a precipitation of salts on the introduction of the sugar solution. For this reason it is better to determine the copper in the precipitated cuprous oxid by some such method as Low's.¹ Burning the cuprous oxid to cupric oxid will only correct for the organic matter, so it could not replace an actual copper determination.

A comparison of these methods on samples of commercial feeding stuffs of which molasses was one of the ingredients resulted as follows:

Results on molasses feeds by the two methods.

Serial No.	Alcohol digestion method.		Sodium carbonate digestion method.			
			Lead acetate.		Alum clarification.	
	Reducing sugars.	Total sugars.	Reducing sugars.	Total sugars.	Reducing sugars.	Total sugars.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
7894	3.55	7.90	4.54	8.68	5.98	9.07
7901	4.98	9.86	5.53	10.44	6.15	11.17
7900	2.54	28.82	3.25	32.15	3.41	32.37
7934	5.38	10.09	7.11	11.37	(¹)	(¹)
8049	1.81	33.65	1.88	34.36	(¹)	(¹)
8050	1.15	11.54	1.26	11.89	(¹)	(¹)

¹ No results by this method.

These figures show some peculiar variations. In the sodium carbonate digestion method when lead was used as the clarifying agent the results are lower and more closely approach the alcohol digestion figures. In samples No. 8049 and 8050 the results by the two methods are fairly comparable, but in the others, and especially in No. 7900, the sodium carbonate digestion method gives much higher figures for the percentage of total sugars, while in Nos. 7894, 7901, and 7934 the increase in total sugars is not so large as the increase in reducing sugars. The original samples left were not large enough to determine the cause of this increase. For sample No. 7900 the increase in total sugars amounts to 3.33 per cent. The results on this sample were carefully checked and the determination was also made by the section of plant physiological chemistry and the results as shown in the table agreed with those obtained by the sugar laboratory. A portion of the sample was placed in a Soxhlet extractor and extracted with alcohol until no sugar was found in the alcohol by the α -naphthol test. Another portion of the sample was boiled for two and a half hours with a measured quantity of water and the sugar determined in this extract. Little or no starch was present in this

¹ U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised, p. 241.

sample, so that this method could be used. The results of this work are given in the following table:

Check analyses of sample No. 7900 (molasses feed), using several methods.

Methods.	Reducing sugars.	Total sugars.
	<i>Per cent.</i>	<i>Per cent.</i>
Alcohol digestion method ¹	2.54	28.82
Do. ²	2.81	28.98
Do. ³	3.10	29.54
Alcohol extraction (Soxhlet extractor) ⁴	2.75	29.24
Sodium carbonate digestion method ¹	3.41	32.36
Do. ²	3.52	31.74
Do. ³	3.28	32.11
Hot water digestion ³		32.44
Do. ²	2.94	31.68

¹ Results by Mr. Given.

² Results by Mr. Straughn.

³ Results by Mr. Jacobs.

⁴ Results by Mr. Bryan.

The results obtained with alcohol, either by digestion or by extraction, agree among themselves, as do also the results with hot water and cold sodium carbonate digestion, but comparing the figures obtained when water was the extracting agent with those obtained when alcohol was used, there is noted an increase of over 3 per cent in total sugars extracted by water. Unfortunately after this work was done none of the sample was left on which to study the cause of this difference. It might result (1) because the alcohol did not extract all the sugars; (2) because the sodium carbonate did not inhibit the action of the enzymes; (3) because the cuprous oxid was badly contaminated in the sodium carbonate digestion; (4) because the sodium carbonate method extracts substances which reduce Fehling solution, especially on inversion, that are not extracted by alcohol.

The first suggestion is answered by the fact that extraction in a Soxhlet extractor was run to a point at which no reaction for sugar with α -naphthol was given and the results of this method were practically the same as those obtained by the alcohol digestion method. Also in experiments using 12 grams of sucrose as the material for extraction by the alcohol method in one test and 12 grams of invert sugar in another, there was no sugar remaining undissolved and no sugar was precipitated by the addition of 95 per cent alcohol to the 300-cc mark. This shows that the percentage of sucrose and invert sugar up to 100 per cent would be soluble in the alcohol treatment. The second explanation was shown to be unsatisfactory by extracting the material with boiling water; the results obtained were practically the same as by the sodium carbonate extraction method in the cold and, therefore, the activity of enzymes could not have caused the difference.

The third proposition is refuted by treating the crucibles coming from some of these determinations with nitric acid and determining the copper by Low's method.¹ Calculating this copper to invert sugar, the results were somewhat lower, but practically the same difference remained between the results by the two methods. Having eliminated the other possibilities the cause of the difficulty seems to narrow down to the nature of the substances extracted by the two solvents. Water removes from vegetable matter together with sugars many other substances, such as gums, pentosans, and some glucosids. These as a rule are more soluble in water than in 50 per cent alcohol and some are rather easily hydrolyzed by acids, yielding reducing sugars. From the previous table it is seen that the principal increase occurs in the total sugars or in the sugars after inversion, and not so much so in the sugars before inversion. A new sample of this particular molasses feed (No. 8049, p. 7) was obtained from the manufacturer with a view to studying this point further, but the results on this sample showed such slight differences by the two methods that no further work was deemed necessary. In the case of molasses feeds it is of extreme importance to have the sample finely ground before extraction. Especially is this so when using the alcohol digestion method, as the alcohol tends to hold back gummy material and this might coat the larger particles of the sample and keep them from being extracted. Molasses feeds are difficult to grind at the best, as they cake very readily, but by first drying carefully they can be finely ground.

Having compared the results obtained by the two methods on representative samples, it now remains to study the limitations of these methods.

LIMITATIONS OF THE METHODS.

SODIUM CARBONATE DIGESTION METHOD.

The purpose of the addition of sodium carbonate is to inhibit enzymes. Should the material under extraction be acid in reaction, then a portion or all of the sodium carbonate will be neutralized, and the necessary amount of sodium carbonate to inhibit enzymes will not be present. It was found that the acidity of one sample of molasses food neutralized about 90 per cent of the sodium carbonate and in two others over 50 per cent was neutralized. Therefore in using this method it is seen that the acidity of the material must be determined and corrected by the addition of more sodium carbonate, otherwise the results obtained would not be correct if the material contained active enzymes.

¹ U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised, p. 241.

As the addition of alum or lead for clarification neutralizes the sodium carbonate, and therefore removes the agent inhibiting the enzymic action, the procedure from this point must be hastened as much as possible to prevent changes in the sugar content. Should the filtration of the clarified solution be difficult and some time be consumed in obtaining a clear filtrate, it is better to throw away the solution and extract the sample again, as the results are very apt to be incorrect. The few results in the following table will show the possible errors in such determinations:

Results showing errors introduced by slow filtration.

Serial No.	Material.	Quick filtration.		Slow filtration and less speed.	
		Reducing sugars.	Total sugars.	Reducing sugars.	Total sugars.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
7959	Wheat.....	0.18	1.73	0.32	2.05
7894	Mixed cattle feed.....	1.50	4.03	1.03	5.06
	Molasses feed.....	5.98	9.07	6.77	10.40

The more time consumed from the point of clarifying to that of determining the reducing sugar, the greater will be the error. Determinations were made on the fresh extract and on the extract that had stood overnight after clarification.

A few of the results are given in the following table:

Data showing effect on results of standing overnight.

Serial No.	Sample No.	Immediate determination.		Standing overnight.	
		Reducing sugar.	Total sugar.	Reducing sugar.	Total sugar.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
7856-B.	1.....	0.23	1.86	1.66	3.83
7856-C.	2.....	.38	2.42	1.72	2.22
7856-A.	3.....	.42	3.30	2.41	3.73
7893	4 Corn meal.....	.17	1.98	1.23	2.25
7894	5 Molasses feed.....	5.98	9.05	10.41	11.50
8207	Alfalfa ¹	1.58	1.60	2.80	2.63
8209	Alfalfa and corn ¹	1.97	3.86	3.65	4.90

¹ Lead acetate as a clarifier.

Standing overnight in all cases greatly increased the reducing sugar content. In some instances this increase was over 500 per cent. The total sugars in all except one case (No. 7856-C) showed a marked increase, but the percentage increase is far below that of the reducing sugars. From the last two tables it is seen that allowing the solution to stand for any length of time after clarification increases the percentage of sugars present. This increase is due, no doubt, to enzymic

action; the inhibiting agent having been neutralized, the enzymes again become active.

Time is an important factor in chemical analysis, as a chemist is often called away from his work and the solution in consequence stands for some time. It is well, therefore, to have a method that will allow of such delays without seriously affecting the results. When using the sodium carbonate digestion method such a lapse of time will certainly introduce an error, and it is readily seen that only the results which have been obtained by following very closely the procedure as outlined can be relied upon.

ALCOHOL DIGESTION METHOD.

The limitations of the sodium carbonate do not seem to apply in the case of the alcohol method. No substance need be added to the alcohol to destroy or inhibit the enzymes, as the alcohol itself and also the heat of extraction effects this. Hudson and Paine¹ have lately found that the enzym invertase is destroyed by 50 per cent alcohol. After the enzymes are killed there is little chance of the extract changing on standing except through outside contamination. Church and Straughn² in their corn work have many times analyzed a portion of the fresh alcoholic extract and then shipped the remainder of their extract to be examined later. Results in all cases have been comparable, and the samples in some cases have been stored for from four to eight months before analysis. The alcohol method as given on page 3 indicates two or three points at which the work can be stopped and as much as a day or more elapse without fear of introducing any error. This is of great importance to the chemist who is called upon to make other determinations at the same time and who is liable to be called away from his work at any time. The natural acidity of the sample in this method does not play as important a part as in the other one. It is obvious, however, that if digestions of strongly acid substances are to be made the material should be neutralized. Again, if the alcohol used is strongly acid, it also should be neutralized.

DUPLICATING RESULTS.

Some methods will yield duplicate results when the determinations are carried on side by side that may not give concordant results when used at another time on the same sample. This is often noted when uncontrollable conditions which affect the results are present, or when the details of the manipulation are not accurately described in the method. When an extraction is to be made and the extract analyzed, questions of temperature and length of time of

¹ U. S. Dept. Agr., Bureau of Chemistry Cir. 58.

² U. S. Dept. Agr., Bureau of Chemistry Bul. 127.

extraction are important. The latter point is generally taken into account by chemists, but temperature is not always considered unless special note is made of it. Directions to conduct extractions at room temperature are rather vague, for this temperature during the summer varies from that during the winter; and, again, some chemists prefer to work at a much lower temperature during the winter than others, and this plays an important part in the process. However, in the sodium carbonate method, the time, two and one-half hours, with occasional shaking, has been found long enough to remove the sugars under nearly all temperature conditions.

When using the greatest of care with the sodium carbonate method the results of determinations made at different times on the same sample seem to show a fair degree of agreement, as shown by the following results:

Duplicate analyses made at different times by the sodium carbonate method.

Serial No.	Number of analysis.	Reducing sugar.	Total sugar.
		<i>Per cent.</i>	<i>Per cent.</i>
7856-A	First.....	0.24	3.28
	Second.....	.23	3.07
	Third.....	.51	2.91
7856-B	First.....	.23	1.86
	Second.....	.38	1.94
7900	First.....	3.41	32.36
	Second.....	3.52	31.74
7934	First.....	7.11	11.37
	Second.....	6.57	11.46
8049	First.....	1.88	34.36
	Second.....	2.16	35.79
8207	First.....	1.40	1.48
	Second.....	1.58	1.60

The results by the alcohol method show possibly a little better agreement, as given in the following table:

Duplicate analyses made at different times by the alcohol method.

Serial No.	Number of analysis.	Reducing sugar.	Total sugar.
		<i>Per cent.</i>	<i>Per cent.</i>
7856-A	First.....	0.00	2.99
	Second.....	.00	2.93
	Third.....	.00	3.00
7900	First.....	2.54	28.82
	Second.....	2.81	28.97
7934	First.....	5.72	10.12
	Second.....	5.82	10.47
8049	First.....	1.81	33.65
	Second.....	1.73	33.32
7959	First.....	1.46	3.91
	Second.....	1.57	3.99
	Third.....	1.55	4.15

TERMS USED IN STATING RESULTS.

As bearing on the general subject of sugar determinations, a word should be said regarding the methods of stating sugar results on such products as cattle feeds and grains. Some chemists calculate the reducing sugars to dextrose and some to invert sugar, while others state the reducing power in terms of metallic copper reduced by a given weight of the material under examination. The expression of the results in terms of sugars is more definite than in terms of copper reduction, which gives no clear idea of the amount present. Indeed, it is doubtful whether the reducing action noted is due to one particular sugar; it may be due to a mixture of several sugars or to invert sugar which is a mixture of equal parts of dextrose and levulose. In only a very few cases have the sugars been separated and identified. Therefore, for general work it seems that sugars before inversion should be reported as "Reducing sugars calculated as dextrose" or "Reducing sugars calculated as invert sugar," depending on which calculation was made. And for sugars after inversion the same phraseology should be used, namely, "Total sugars calculated as dextrose" or "Total sugars calculated as invert sugar." The increase in reducing sugars after inversion may not be caused entirely by the inversion of sucrose, but may be due to other compounds or sugars being split up into reducing sugars by the acid. It is well known that the ordinary inversion methods will hydrolyze other sugars, for instance, raffinose, and may hydrolyze such compounds as inulin or some of the glucosids and pentosans, forming reducing sugars. Under such circumstances it is certainly wrong to calculate the increase in reducing sugars as sucrose without a more definite knowledge of these sugars, although a part of this increase may be due to sucrose.

In order to eliminate a number of these compounds and restrict the increase to one or two sugars, the invertase inversion method proposed by Hudson¹ should be used conjointly with the acid inversion. Under such circumstances, should the quantity of total sugars agree by both methods, one may safely say, with our present knowledge, that the increase in reducing sugars is due to sucrose, raffinose, or both. As the latter sugar is present in notable quantities in cotton-seed meal, and may be in other materials, it is not altogether safe to calculate this increase as sucrose with the idea that only sucrose is present. By the invertase method many other substances are eliminated. It seems better, therefore, to use the expressions (1) "Reducing sugars calculated as dextrose," or "Reducing sugars calculated as invert sugar;" (2) "Total sugars calculated as dextrose," or "Total sugars calculated as invert sugar," and (3) "Increase in reducing sugars by acid hydrolysis (or by invertase) calculated as sucrose."

¹ U. S. Dept. Agr., Bureau of Chemistry Cir. 50; J. Ind. Eng. Chem., 1910, 2: 143-5.

SUMMARY.

A method of preparing an extract for sugar determinations in grains, cattle foods, and vegetable material in general is given on page 3, depending on boiling the product with 50 per cent alcohol. A comparison of the results obtained by this method using various classes of materials is given with that of a method depending on the extraction of the sugars with water at room temperature to which a percentage of sodium carbonate is added to inhibit enzymic action. A study of the limitations of the two methods is made, in which it is shown that the alcohol method will give satisfactory and comparable results on material, whether it is analyzed immediately or after standing for some time, while to obtain true and concordant results with the sodium carbonate method the most careful attention must be given to certain details of manipulation. In the sodium carbonate method, after neutralizing the sodium carbonate by clarifying with alum or lead, the work must be carried on with dispatch as the enzymes again become active; (2) the acidity of the sample must be determined and the quantity of sodium carbonate thereby regulated, or the accuracy of the results will probably be affected; (3) normal lead acetate should be used instead of alum for clarification under most circumstances, otherwise an error is introduced. Taking these points into consideration, it is the opinion of the authors that the alcohol method is to be preferred in general sugar work, but when a long, comparable series of results is to be obtained on samples of the same kind of material, the sodium carbonate method might be used advantageously, provided it is run with dispatch and the greatest care is exercised in its operation; and, most important of all, the results obtained by its use should compare with those obtained by alcohol digestion. Some materials, however, can not be analyzed by the sodium carbonate method because clear filtrations are not obtainable even with lead clarification.

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