Colorimetric Micro Determination of Calcium in Sugar-House Products

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Introduction

The quantitative estimation of calcium in sugar-house products, such as raw juice, clarified juice, syrup, molasses and raw sugar is very important in sugar manufacturing. Many methods for this estimation exist in the literature pointing to the importance of the calcium content, but also to the complexity of the problem. Since the standard analytical oxalate method is cumbersome, many routine methods have been proposed. The eldest of these is the soap method of Spengler and Brendel (4)[2] which gives an estimate of the total quantity of calcium and magnesium, with doubtful accuracy, however.

Several workers have developed in recent years more elegant methods based on the EDTA (ethylenediamine tetra acetate) reaction. In these methods the sugar solution is titrated with a solution of EDTA in the presence of eriochrome black [Honig (1), Saunier and Lemaitre (3)]. The amount of EDTA employed is a measure of the calcium plus magnesium content. When the calcium content is desired, a second titration is carried out on the sugar solution after precipitation of the calcium, or another indicator such as murexide [Ramaiah, Vishnu and Chaturvedi (2)] is used, which, however, requires a photometric titration apparatus. The great disadvantage of the EDTA titrations, even with a photometer, is the troubles which occur on examining stark colored solutions like raw juice and molasses. It is significant that in the 1964 edition of the ICUMSA methods of sugar analysis, a method for determination of calcium is not mentioned.

The present communication reports a simple, sensitive and accurate method for estimation of calcium, which is elaborate by Trinder (5) for blood, but so far is not applied to sugar-house products.

Materials and Methods

In the proposed hydroxamate method, the calcium is precipitated as its naph-thalhydroxamate. After centrifugation, the unwashed precipitate is dissolved and the color intensity of the

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[2] Numbers in parentheses refer to literature cited.

obtained solution after addition of ferric nitrate is measured in a simple colorimeter.

Reagents—Calcium reagent solution made by dissolving (under heat) 20 mg naph-thalhydroxamic acid in 100 ml of water containing 5 ml of ethanolamine and 2g of D tartaric acid. After cooling, 9g of sodiumchloride dissolved in water is added and diluted with water to 1 liter.

Alkalin solution is made by dissolving 2g EDTA (disodium-salt) in 1 liter of 0.1 N sodium hydroxide.

Color solution is made by dissolving 60g ferric nitrate nine hydrate in 1 liter of water containing 15 ml nitric acid (D = 1.4).

Standard Calcium solution is made by dissolving 100.1 mg analytical reagent dry calciumcarbonate in 0.1 N hydrochloric acid and diluting to 1 liter with water.

Procedure—0.5 ml of sugar solution is mixed in a centrifuge tube with 5 ml of calcium reagent solution and after 30 minutes is spun in an ordinary laboratory centrifuge for 5 minutes. The supernatant liquid is carefully poured off and, while the tube is still inverted, it is placed in a rack to drain on filter paper. After a few minutes, the mouth of the tube is wiped dry with filter paper and 1 ml of alkaline solution is added. The tube is covered with a glass stop or an aluminium cap and heated in a boiling waterbath, shaking the tube at intervals to ensure complete solution of the precipitate. After 10 minutes, the tube is removed and when cool 3 ml of the color solution is added and the content of the tube mixed. The optical density of the solution is then measured at 450 mμ against a blank prepared by carrying out the same procedure, but with omission of the sugar solution. The calcium content of the sugar solution is calculated from a graph, obtained with standard calcium solutions containing 4 to 40 μg calcium in the 0.5 ml, analyzed together with the test.

The above procedure is carried out on undiluted raw and clarified juices, while syrup and molasses samples were diluted to a concentration of about 20 μg calcium in 0.5 ml. In order to study the possible influence of organic matter and complexes, the above procedure is simultaneously carried out on the samples after digestion with a mixture of concentrated nitric acid and perchloric acid.

The results obtained with the hydroxamate method were compared with those obtained with the standard oxalate method. Herewith, the calcium in the sugar solution, digested or not, is precipitated at pH 5 with saturated ammonium oxalate. After

centrifuging, washing with ammonia water and again centrifuging, the precipitate is dissolved in warm dilute sulfuric acid and titrated with 0.01 N potassium permanganate.

Results and Discussion

The results obtained with the hydroxamate method and with the oxalate method on 5 samples of raw juice, 3 samples of clarified juice, 3 samples of syrup, 3 samples of molasses and 1 sample of raw sugar, with and without digestion, are mentioned in Table 1. In order to establish if the difference between the calcium content found with the four methods is significant,

Table 1.—Calcium content (mg/ 100° Brix) in various sugar-house products, obtained by different methods.

Sample	Without digestion				With digestion			
	Hydroxamate 1		Oxalate 2		Hydroxamate 3		Oxalate 4	
	Content	Reprod.	Content	Reprod.	Content	Reprod.	Content	Reprod.
Raw juice	31.2	2.1	23.5	4.1	35.6	4.0	26.0	8.2
Raw juice	18.1	0.9	7.7	3.0	19.5	3.9	14.7	2.8
Raw juice	19.8	5.1	9.1	10.8	17.2	9.9	9.7	11.2
Raw juice	10.9	5.7	2.6	14.6	11.4	3.8	7.5	1.6
Raw juice	32.7	3.4	22.0	2.5	34.3	0.8	26.5	0.8
Mean	22.5	3.4	13.0	7.0	23.6	4.5	16.9	4.9
Clar. juice	27.9	0.9	29.9	1.2	30.4	4.4	28.5	2.1
Clar. juice	40.3	3.1	40.5	0.6	36.0	0.6	42.0	1.5
Clar. juice	24.9	2.9	27.4	0.3	28.7	0.6	28.8	1.5
Mean	31.0	2.3	32.6	0.7	31.7	1.9	33.1	1.7
Syrup	35.5	4.1	37.2	0.7	34.8	1.7	37.9	1.8
Syrup	17.7	1.0	17.4	1.2	16.9	2.4	17.7	3.1
Syrup	12.9	4.3	13.9	1.3	15.7	4.2	13.6	11.5
Mean	22.0	3.1	22.8	1.1	22.5	2.8	23.1	5.5
Molasses	180.7	3.3	202.5	4.8	183.2	0.8	192.4	1.6
Molasses	92.7	3.1	89.2	1.3	96.6	10.2	92.7	3.0
Molasses	83.0	0.6	69.6	2.2	92.9	2.7	90.4	1.1
Mean	118.8	2.3	120.4	2.8	124.2	4.6	125.2	1.9
Raw sugar	9.6	4.8	9.5	1.1	11.0	3.7	10.1	3.5
General Mean	42.5	3.0	40.1	3.3	44.3	3.6	42.6	3.7

Table 2.—P levels at which the difference between the mean calcium content, obtained with four methods, is significant.

Samples \ Methods	1 - 2	1 - 3	1 - 4	2 - 3	2 - 4	3 - 4
Raw juice	0.005	0.4	0.01	0.005	0.02	0.005
Clar. juice	0.2	0.8	0.2	0.7	0.7	0.6
Syrup	0.3	0.8	0.3	0.8	0.5	0.7
Molasses	0.9	0.2	0.2	0.8	0.7	0.8
All samples	0.3	0.1	0.9	0.1	0.2	0.2

the t test is applied and the values obtained are mentioned in Table 2. From these two tables, it appears that the mean calcium content found on the raw juice with the four methods is 22.5 - 13.0 - 23.6 and 16.9 mg/100° Brix respectively. These four values differ significantly in all combinations, except for the hydroxamate method on the digested and undigested samples. It is striking that the results obtained with the hydroxamate method on the digested and undigested samples are significantly no different, while digestion increases significantly the calcium content found with the oxalate method. In some cases the content increases with digestion 2 to 3 times and reaches almost the values found with the hydroxamate method.

The mean values for the calcium content of the clarified juices, obtained with the four methods are 31.0 - 32.6 - 31.7 and 33.1 respectively, which are not significantly different. The same conclusion is valid for the mean calcium content of the syrup and the molasses samples, which have the values 22.0 - 22.8 - 22.5 - 23.1 and 118.8 - 120.4 - 124.2 and 125.2 mg/100° Brix respectively. Summarizing it may be concluded that, except for the raw juice samples, the results obtained with the hydroxamate method do not differ significantly from those obtained with the oxalate method, whether or not the samples are or are not digested.

In regard to the precision or the reproducibility of the methods applied, the analysis on each sample is made in triplicate and the coefficient of variation as a measure of the reproducibility is calculated by dividing 100 times the standard deviation obtained with each sample by the mean of the three results. From the results mentioned in Table 1 and 3 it appears that the reproducibility does not significantly differ between the four methods applied on the different samples, except for the oxalate method applied on digested and non-digested clarified juice samples. The co-efficient of variation obtained with the hy-

Table 3.—P levels at which the difference between the coefficient of variation, obtained with the four methods, is significant.

Methods / Samples	1 - 2	1 - 3	1 - 4	2 - 3	2 - 4	3 - 4
Raw juice	0.1	0.5	0.5	0.3	0.5	0.7
Clar. juice	0.2	0.8	0.6	0.4	0.01	0.9
Syrup	0.2	0.8	0.5	0.1	0.3	0.4
Molasses	0.8	0.5	0.6	0.7	0.6	0.4
All samples	0.8	0.5	0.5	0.8	0.8	0.9

droxamate and with the oxalate method applied on the 15 undigested and digested samples attains the value of 3.0 - 3.3 - 3.6 and 3.7 respectively, which are only slightly different. From all these results it can be concluded that there are no great differences between the reproducibility of the four methods applied.

Since the hydroxamate method gives results which are not significantly different from these obtained with the oxalate method and with a practical equal precision, this method may be recommended. The hydroxamate method is much less time consuming than the oxalate method and at least 25 times more sensitive. The hydroxamate method can be applied on the various sugar-house products without digestion or defecation, even on raw juices, which is not the case for the oxalate method. It is interesting to remark here that the complexometric titration with eriochrome black or with murexide is also much less sensitive than the hydroxamate method and gives troubles with dark solutions like raw juice and molasses. With these solutions it is necessary to clarify with lead reagent, with subsequent precipitation of the excess of lead. With molasses this procedure is even not sufficient and decolorization with adsorptive carbon is necessary.

All these cumbersome manipulations are superfluous with the hydroxamate method, which is so sensitive that the molasses may be diluted to 2° Brix and the resultant color has no influence on the precipitated calcium. The simple complexometric titration on clarified juice is somewhat less time consuming than the hydroxamate method, but this advantage disappears when defecation and decolorization is necessary. Finally, a high sensitive method like the hydroxamate method is very interesting on examining samples with low calcium content like refined sugars. It is our conviction that the hydroxamate method is a reliable and convenient method and may be successfully applied in the sugar industry.

Summary

A simple colorimetric micro method for calcium determination in sugar-house products is described. The method consists essentially in a precipitation of the calcium with naphthalhydroxamic acid, which is separated by centrifuging, dissolved in an alkaline solution, after which the color reaction with ferric ions is measured in a simple colorimeter. From the statistical calculations, it appears that the hydroxamate method

gives no significantly different results in comparison with the standard oxalate method and with practical equal precision or reproducibility. Since the hydroxamate method is more sensitive and less time consuming than the oxalate or complexometric method and can be carried out directly on the samples without digestion or defecation, even on raw juices or molasses, it is a reliable and convenient method, which can be successfully applied in the sugar industry.

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