

A Research Note

THE QUANTITATIVE DETERMINATION OF GLUCOSE, FRUCTOSE AND SUCROSE IN FRUITS AND POTATOES

INTRODUCTION

IN PROCESSED FRUITS and vegetables the brown coloration, off-flavors, excessive hygroscopicity and other undesirable properties often result from the interaction of natural sugars with other reactive components in these foods. Consequently, in food processing studies, determination of the major sugars is essential at various stages, e.g., as the fresh product before and after storage conditioning, and in the final processed form.

Most sugar analyses are based on the oxidation-reduction reaction involving alkaline solutions of metal salts. Ting (1956) utilized the reduction of alkaline ferricyanide to determine total reducing sugars in citrus juices. Furuholmen et al. (1964) modified Ting's method and applied it to the determination of glucose and fructose in potatoes. The difference in the rates of reaction at 55° and 100°C of the monosaccharides with potassium ferricyanide was utilized. Although this method is satisfactory for measuring the total monosaccharide concentration when reacted at 100°C, it does require rigid control of temperature at 55°C to differentiate between glucose and fructose. Therefore, the highly specific glucose oxidase method described by White (1964) for determining glucose was used to supplement the total reducing sugar analysis.

Jacobs (1951) and Schwimmer et al. (1954) have indicated that the major sugars in fruits and potato tubers are glucose, fructose and sucrose. Therefore, the fructose content can be estimated as the difference between total reducing sugar and glucose concentration. Sucrose has been determined by measuring the increase in glucose content after acid hydrolysis of the disaccharide.

MATERIALS & METHODS

A HITACHI Perkin-Elmer, Model 139, UV-Visible Spectrophotometer and a Beckman, Model B, Spectrophotometer were used to measure absorbance.

The alkaline ferricyanide and arsenomolybdate (A.R. Grade) solutions were prepared as described by Ting (1956).

Glucose oxidase-peroxidase reagent was prepared (White and Subers, 1961) using either

crude or the purified-Type II glucose oxidase (Sigma Chemical Co.) and Type I, horseradish peroxidase (Sigma Chemical Co.).

Neutral lead acetate solution was prepared by dissolving 20g Pb(OAc)₂ · 3H₂O in de-ionized water to a final volume of 100 ml.

Reagent grade glucose, fructose and sucrose dried under vacuum at 70°C for 18 hr were used as standards. A standard stock solution containing 5.000g of glucose per liter was standardized iodometrically (Browne and Zerbán, 1941). Working standards, ranging from 50–500 µg glucose per ml, were used for standardization of both methods.

Total reducing sugars (glucose plus fructose) were determined by reacting with alkaline ferricyanide at 100°C for 10 min (Furuholmen et al., 1964). Absorbance was measured at 720 nm 40 min after addition of arsenomolybdate complexing agent. Both sugars in the concentration range 0–400 µg gave highly significant linear functions ($r = 0.9996$) with an absorptivity of 17.20 for a 1% solution. Periodic checks showed excellent reproducibility of the absorptivity value.

Samples were then analyzed specifically for glucose using the glucose oxidase procedure as described by White (1964). 200 µg of glucose had an absorptivity of about 0.423 when reacted under the above conditions. Although a highly significant linear function ($r = 0.9995$) was obtained in the concentration range studied (0–400 µg), the absorptivity varied sufficiently to necessitate a daily check of the standard value. This value was used to calculate the glucose concentration. The fructose content was calculated as the difference between the total reducing sugar value and the glucose content.

Finally, the sucrose in a measured aliquot of each sample was hydrolyzed with 5 ml 12N HCl at 70°C for 10 min, cooled and neutralized with 5N NaOH. The solution was then re-assayed for glucose by the glucose oxidase reaction. The increase in glucose was used to calculate the concentration of sucrose.

Fresh products, e.g., apples, peaches and potatoes were prepared for analysis by homogenization and filtration of the resultant puree. The filtrate (juice) was clarified with neutral Pb(OAc)₂, diluted and analyzed as described. Partially dried or dehydrated fruits and fruit juice powders were rehydrated with hot de-ionized water to their original moisture level and treated as the fresh product. With processed potatoes ethanol extraction was utilized.

RESULTS & DISCUSSION

THE RELIABILITY of the analytical procedure was tested with a series of ternary mixtures containing varying amounts of glucose, fructose and sucrose. The total sugar content of each mixture was determined and the results (Table 1) were compared to the known composition. The average overall recovery of the added sugars was satisfactory. The glucose content averaged 100.19% (+1.99 to -0.70%) of the amount added. The fructose accounted for averaged 100.81% for all five mixtures, but showed a slightly greater range (+3.83 to -1.30%) than did glucose. Sucrose content from the five mixtures averaged only 97.66% with considerably more variation (+8.25 to -9.50%) than the two monosaccharides. The generally good overall recovery of added sugars indicates this to be a workable method for determining glucose, fructose and sucrose simultaneously.

The suitability of hot water rehydration for the quantitative extraction of sugars from dried fruits was ascertained. In this experiment the sugar content of a partially dried apple was compared to the sugar content of the fresh product from which it was prepared. The results

Table 1—The recovery of sugars from ternary mixtures

Mixture	Glucose mg/100 ml		Fructose mg/100 ml		Sucrose mg/100 ml	
	Added	Found	Added	Found	Added	Found
1	601	602	1000	1025	201	190
2	201	205	600	623	200	181
3	1000	993	1000	1000	100	100
4	201	201	200	198	2000	2165
5	1000	995	1000	987	1001	951

Table 2—Efficiency of extraction procedure for removing sugars from potatoes

Sample	Extraction method	Glucose	Fructose	Sucrose
		Percent ^a		
Fresh potato	Squeezed juice	1.64 ± 0.03	1.70 ± 0.07	2.50 ± 0.03
	Soxhlet	1.49 ± 0.04	2.23 ± 0.01	2.65 ± 0.11
	AOAC	1.49 ± 0.01	1.98 ± 0.13	2.34 ± 0.15
Dried potato	Soxhlet ^b	1.24 ± 0.00	4.02 ± 0.26	1.52 ± 0.01
	AOAC ^b	1.26 ± 0.01	3.46 ± 0.25	1.34 ± 0.14
	AOAC ^c	1.22 ± 0.10	3.50 ± 0.33	1.04 ± 0.01

^a Uncorrected for moisture content

^b Potato rehydrated prior to extraction with 95% ethanol

^c Potato extracted directly with 80% ethanol without rehydration

showed that 97% of the glucose and slightly more than 100% of the fructose and sucrose were recovered. Hot water rehydration was found equally reliable when applied to processed peaches and various fruit juice powders.

When applied to processed potatoes hot water extraction was not feasible because of water retention by the gelatinized starch. Ethanol extraction has been utilized for the removal of sugars from plant materials (AOAC, 1970). However, the suitability of this method for quantitatively extracting the total sugars from processed potatoes at any moisture level was uncertain. The efficiency of ethanol extraction was therefore evaluated by comparing the sugar content of fresh potato analyzed "as is" and the same potato extracted with ethanol. In this study a Soxhlet extractor was employed. For comparison the AOAC extraction method, although somewhat troublesome because of bumping and occasional boil-over, was also used. In each extraction sufficient 95% ethanol was added to fresh potatoes to yield a final ethanol concentration of 80% when diluted with the sample moisture. After extracting for 90 min the mixture was cooled and filtered into a volumetric flask. The extracted potato sample was washed several times with 80% ethanol and the washings added to the original extract. Clarification of this solution was unnecessary. Due to the inhibitory effect of ethanol on the glucose oxidase reagent, however, it was necessary to remove all traces of alcohol from the samples using a rotary vacuum evaporator prior to glucose determina-

tion. The results of this comparative study are shown in Table 2. The sugar content of the potato juice, assumed to represent the true sugar content of the sample, was used as the basis for comparison with the ethanol extracts. The average concentration of glucose, fructose and sucrose in the fresh potato samples extracted with ethanol are of the same order of magnitude as the concentration of these sugars in the control (potato juice) sample. Thus, the two procedures appear equally reliable for extracting sugars from fresh potato.

Although the Soxhlet extraction was suitable for removing sugars from fresh potato, erratic results were obtained when tried on dried potato dice (4% moisture). The problem was the absence of sufficient sample moisture to dilute the alcohol-water azeotrope (95%–5%) which is formed during reflux. The solubility of sugars is greatly reduced in 95% ethanol, thus limiting extraction efficiency. Prior to extraction, therefore, sufficient de-ionized water was added to the ground dried potato sample increasing the moisture level to that of fresh potato (85–90%). Sufficient water is then present to dilute the azeotrope, especially at the early stages of refluxing, thereby insuring maximum solubility of the sugars.

Table 2 also shows the glucose, fructose and sucrose concentrations of a typical sample of dried potato dice extracted by the modified Soxhlet procedure described above. Also shown are the sugar concentrations of the same sample that was extracted by the direct method (AOAC, 1970) both with and without

prior rehydration. The glucose content of the dried potato extracted by the three procedures is identical. Fructose and sucrose levels are slightly higher in the Soxhlet extract. It appears that sucrose solubility may be enhanced by preliminary rehydration of the dried sample prior to extraction by either procedure.

SUMMARY

THE UTILITY of the Soxhlet extraction technique for removing sugars from fresh potato with 80% ethanol has been shown. The procedure is equally effective for extracting sugars from processed potato provided the potato sample is rehydrated prior to ethanol extraction.

Hot water extraction of dried fruits and fruit juice powders has been shown to be adequate for extracting sugars from these products.

Ternary mixtures of glucose, fructose and sucrose can be analyzed by a two-step procedure utilizing the determination of total reducing sugars coupled with the use of the glucose oxidase reaction for measuring glucose specifically.

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