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Registry No. Gln, 56-85-9; Asp, 70-47-3; Lys, 56-87-1; Arg, 74-79-3; His, 71-00-1; Glu, 56-86-0; Leu, 61-90-5; Pro, 147-85-3; Val, 72-18-4; N, 7727-37-9.

Determination of Dextrose Equivalent in Starch Hydrolysates Using Cerium(IV)

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The time for reduction of a fixed amount of Ce(IV) by an excess of carbohydrate solution (4.0% w/v) was dependent on the concentration of reducing sugar in the solution. The colorimetric conversion of Ce(IV) to Ce(III) could be observed visually or, for more accurate determinations, with a spectrophotometer set to monitor the absorbance change at 445 nm. The inverse of the Ce(IV) reduction time gave an excellent linear correlation ($r = 0.995$) with the dextrose equivalent (DE) of starch hydrolysate solutions as measured by using the Lane Eynon method. The Ce(IV) test was simple, rapid, and inexpensive and offered an alternative method for the determination of DE in process control of starch hydrolysis.

Although time-consuming, the Lane Eynon or similar copper complex oxidation–reduction reactions have been the traditional and official methods for measuring the dextrose equivalent (DE) of starch hydrolysates (De Whalley, 1964). DE is an important property of starch hydrolysates, being an indirect measure of the degree of hydrolysis of the starch. Higher hydrolyzed products are sweeter and more soluble in water and have different effects upon the rheology and texture than products of lower starch hydrolysis. A product with a high DE has a higher degree of hydrolysis than a product with a lower DE. Numerous other methods, notably, freezing point depression (cryoscopy) and high-performance liquid chromatography (HPLC), have been introduced as possible replacements for the traditional quality control methodology (Delheye and Moreels, 1988). Although rapid, cryoscopy requires relatively expensive equipment and is affected by a variety of production parameters such as the raw material, production method, purification technique, and salt content. HPLC gives quantitative information on oligosaccharides present but also requires expensive equipment, greater expertise, time, and conversion of each oligosaccharide with a factor to establish the overall DE of the hydrolysate.

While working on a method for measuring lactose hydrolysis in milk, Griffith et al. (1989) noted that hydrolysis to monosaccharides could be measured by determining the increasingly rapid reduction of cerium(IV) to cerium(III) using an excess of carbohydrate. This paper investigates the use of this cerium reaction to measure the DE of starch hydrolysates.

EXPERIMENTAL PROCEDURES

Materials. Carbohydrate standards (glucose and glucose oligosaccharides) were purchased from Sigma Chemical Co., St. Louis, MO. Starch hydrolysate materials were provided courtesy of American Maize Products Co., Hammond, IN; A. E. Staley Manufacturing Co., Decatur, IL; and Champlain Industries Ltd., Mississauga, ON. Water was prepared by using a Millipore Milli-Q system, and all other chemicals used were of reagent grade or better.

Ce(IV) Oxidations of Carbohydrates. Oxidations were carried out in a manner similar to the method reported by Griffith et al. (1989) except that the time in seconds was recorded for the absorbance at 445 nm to return to 0.5 OD rather than 0.4 OD. The rationale for the choice of 445 nm is explained in the earlier work of Griffith et al. (1989).

The method involved addition of 0.4 M ammonium hexanitratocerium(IV) [$(NH_4)_2Ce(NO_3)_6$] in 0.5 M nitric acid to carbohydrate solution in a 1:3 ratio. The Ce(IV) solution was aged at least 6 h and shaken before use. The carbohydrate solution was prepared at a concentration of 2.00 ± 0.01 g in 50 mL of water, or equivalent (for carbohydrate standards, less carbohydrate in proportionately less water was used). The mixed solution was placed in a Pye-Unicam SP 1800 spectrophotometer (sugar solution as reference sample) and the time in seconds

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Table I. Time for Aqueous 4.0% Carbohydrate Solutions To Reach an Absorbance of 0.5 OD at 445 nm after Ce(IV) Addition

carbohydrate	theoretical DE	time,[a] s (±SD)
glucose	100	29.02 ± 0.50
maltose	52.63	49.85 ± 0.42
isomaltose	52.63	49.19 ± 0.42
maltotriose	35.71	68.80 ± 0.63
maltotetraose	27.03	78.84 ± 0.02
maltopentaose	21.74	116.56 ± 0.27
maltohexaose	18.18	176.42 ± 1.35
maltoheptaose	15.63	208.81 ± 1.01

[a] All times are the means of duplicate analyses with the exception of times given for the first three carbohydrates, which were the means of triplicate analyses.

Table II. Time for Aqueous Starch Hydrolysate Solutions To Reach an Absorbance of 0.5 OD at 445 nm after Ce(IV) Addition

product	DE[a]	% moisture	time, s[b] (±SD)	(1/time) × 1000
Lo-Dex 10	11.2	7.1	950.6 ± 6.4	1.05
			945.5 ± 2.1	1.06
Fro-Dex 25	24.6	5.7	155.1 ± 3.4	6.45
			158.2 ± 0.4	6.32
Fro-Dex 24D	28.6	5.5	113.5 ± 1.1	8.81
			114.1 ± 2.3	8.76
Fro-Dex 36P	35.1	5.4	83.3 ± 0.6	12.00
			83.8 ± 0.6	11.93
Fro-Dex 42F	37.8	5.1	76.2 ± 0.6	13.12
			75.7 ± 0.8	13.21

[a] Lane Eynon values. (Determined upon these samples, not averages.) [b] Means of triplicate analyses.

recorded for the absorbance at 445 nm to return to 0.5 OD. The Pye-Unicam spectrophotometer sample compartment was temperature controlled and maintained at 25.1 ± 0.1 °C by using a Lotemptrol 154 bath (Precision Scientific Co.) containing ethylene glycol/water (50:50 v/v).

Percentage Moisture of Starch Hydrolysates. When not given by the starch hydrolysate supplier, the percentage moisture for the starch hydrolysates was determined by using AOAC (1984) Method 31.006.

RESULTS AND DISCUSSION

To properly appraise the potential use of Ce(IV) oxidimetry for measurement of DE in starch hydrolysates, different glucose oligosaccharides were first examined (Table I). At a 4.0% (w/v) concentration, as the degree of glucose polymerization in the oligosaccharides increased, there was also an increase in time required to reduce the absorbance (445 nm) of the colored complex formed between Ce(IV) and carbohydrates as observed previously (Griffith et al., 1989; Virtanen et al., 1987). Although the times increased, even the largest oligosaccharide, maltoheptaose, required only about 3.5 min for completion of the reaction, and both disaccharides of glucose (maltose and isomaltose) required almost the same time. This experiment illustrated that the Ce(IV) oxidation was dependent on the concentration of reducing sugar in a manner very similar to the traditional copper complex oxidation–reduction reactions.

Of course starch hydrolysates represent a mixture of different oligosaccharides of glucose. Therefore the next set of experiments (Table II) was conducted to see if the time for the Ce(IV) oxidations could be correlated in some manner with DE in these products. The starch hydrolysate samples supplied by American Maize Products came with detailed unbiased analyses by the company upon the small amount of samples that were supplied for, among other factors, DE calculated by using the Lane Eynon method and percentage moisture. The Ce(IV) oxidation times of these products at a 4.0% (w/v) concentration were compared to the accurate DE values supplied by the company for the samples furnished for the research to establish the standard curve. Again, times for the solutions to reach 0.5 OD at 445 nm were very consistent, with standard deviations averaging about 1%. Also, duplicate samples from the same batch of hydrolysate gave comparable time values. A plot of DE verses the inverse of Ce(IV) oxidation times gave a linear relationship [DE = 2.16(1/time) + 9.58, correlation coefficient = 0.995]. This was similar to the relationships noted by Griffith et al. (1989) for varying amounts of lactose in solution.

Table III. Comparison of Expected DE Values from Commercial Starch Hydrolysates and DE Values Determined by Using Ce(IV) Oxidimetry

product	expected DE (as given by supplier)	Ce(IV) times,[a] s (±SD)	% moisture	DE calcd[b]
SD 10[c]	10	981.5 ± 25.4	5.2	11.8
SD 20[c]	21.5	208.2 ± 5.5	4.5	20.0
SD 24R[c]	26	115.7 ± 1.9	4.1	28.3
SD 35R[c]	34.5	84.2 ± 2.0	4.1	35.3
SD 42R[c]	43.5	67.6 ± 1.0	3.6	41.6
M100[d]	9.0–12.0	687.0 ± 13.7	5.9	12.7
M200[d]	20.0–23.0	170.7 ± 2.7	6.1	22.2
M250[d]	23.0–27.0	120.0 ± 0.5	4.6	27.6
M550[d]	13.0–17.0	375.5 ± 2.3	6.2	15.3

[a] Means of triplicate analyses. [b] Calculated by using the relationship between DE and inverse of time given in the text. [c] Supplied by A. E. Staley Co. under the trade name Stardri. [d] Supplied by Champlain Industries Ltd. under the trade name Maltrin.

To test the utility of the Ce(IV) oxidation times for determining DE, the starch hydrolysate products from the other two suppliers were tested (Table III). Calculated DE values compared very well with the DE values or range of values reported by the companies for these products. The DE values reported by these companies for their products are typical average DE values for the product and may vary above or below the reported value. The only DE values that were exact for the samples provided were those from American Maize Products because these samples had been accurately analyzed for DE, percentage moisture, and degree of polymerization by HPLC. The companies almost certainly used different techniques for at least some of the processing of their products, and the Ce(IV) oxidation method seemed unaffected by these differences in processing.

If special analytical needs should arise, the Ce(IV) oxidation method lends itself to facile modification to accommodate the needs of the starch producer. For products of low final DE values (less than 10), a faster analysis could be achieved by either lowering the Ce(IV) concentration or increasing the hydrolyzed starch concentration. The simplicity of the test makes partial or total automation possible. Its dramatic color change (red to very pale yellow) makes it possible to also carry out the test visually, although a spectrophotometer as used in this study gives better accuracy. As already found earlier, the oxidation times are affected by temperature (Griffith et al., 1989); thus, temperature changes during the test or deviation from the temperature at which the standard curve was established leads to substantial error. If temperature control is impossible, standard solutions and samples have to be run at the same time, a task most easily done during visual testing.

Finally, the Ce(IV) oxidimetry test was fast, simple, and inexpensive, did not require the use of boiling solutions and rapid titrations (such as the Lane Eynon), and could be modified as required by the processor. There-

fore, the Ce(IV) methodology is an alternative to present methods of determination of DE for starch hydrolysates, especially where required for process control.

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Registry No. Glucose, 50-99-7; maltose, 69-79-4; isomaltose, 499-40-1; maltotriose, 1109-28-0; maltotetraose, 34612-38-9; maltopentaose, 34620-76-3; maltohexaose, 34620-77-4; maltoheptaose, 34620-78-5.

Rapid and Sensitive ELISA Method for the Determination of Bovine Somatotropin in Blood and Milk

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An avidin/biotin ELISA assay for bovine somatotropin (bST) is described. The method uses affinity-purified polyclonal antisera raised in rabbits to immobilize bST from blood or milk samples on the wells of microtiter plates. Bound bST is quantitated by adding biotinylated anti-bST antibody during the sample incubation step, followed by incubations with horseradish peroxidase labeled avidin D and ABTS substrate. Because high-affinity anti-bST antibody is used, and the biotinylated antibody is added directly to the sample, the assay can be performed in less than 4 h while sensitivities of 0.2 and 2 ng/mL in milk and blood, respectively, are maintained. The advantages of time, sensitivity, and simplicity of methodology have made the assay a valuable tool for defining bST concentrations in the blood and milk of dairy cows treated with somidobove, a recombinant form of bST.

Somatotropin is a 22-kDa polypeptide growth hormone that is normally secreted by the pituitary gland. There is substantial evidence to indicate that, among the various direct and indirect biological activities attributed to the hormone, in ruminants somatotropin also mediates a galactogenic effect (Cotes et al., 1949; Brumby and Hancock, 1955; Machlin, 1972). It has been known for many years that milk production in dairy cows can be increased by as much as 20% or more when circulating levels of somatotropin are increased through the administration of exogenous hormone (Hutton, 1957; Peel et al., 1981, 1982, 1983; Bauman et al., 1985; Hart et al., 1985; Mohammed and Johnson, 1985). The potential application of this finding to the management of milk production in dairy herds had, however, been hampered by the cost of obtaining limited amounts of somatotropin available through extraction of pituitary glands. Recently, the application of recombinant DNA techniques to clone and express the gene for bovine somatotropin (bST) has made it possible to obtain an unlimited amount of hormone and provides an opportunity to investigate benefits that may be derived from controlling milk production through supplemental administration of bST on a commercial scale (Spencer, 1987).

Central to studies conducted to investigate the safety and efficacy of recombinant-derived bovine somatotropin (r-bST) treatment was the need for a sensitive analytical method that could be used to estimate bST levels in various biological fluids. An avidin–biotin enzyme-linked immunosorbent assay (ELISA) is described which is sensitive to 0.2 ng/mL in whole milk and 2 ng/mL in blood and can be completed in less than 4 h.

MATERIALS AND METHODS

Materials. Highly purified somidobove (Griffiths, 1988) was obtained from Dr. R. E. Chance, Eli Lilly and Co., Indianapolis, IN, and was used to raise anti-r-bST antibodies in rabbits. Reference Standard somidobove, lots RS0010, RS0041, and RS0071, was obtained from the Analytical Development Division, Eli Lilly. Reference standards had potencies of 1.4 IU/mg, as determined by the rat body weight gain assay. Total bST protein was based on amino acid analysis and nondiscriminatory peptide assays. For purposes of comparison in the ELISA assay, two pituitary-derived bovine somatotropin (p-bST) preparations were used: (1) USDA-bGH-B-1 (AFP-5200) was obtained

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