Determination of % Cook In Extruded Cereal Products Using Chemical Solubilization

I. Introduction

The degree of cook of extruded cereal products can be determined using the YSI 2700 SELECT Biochemistry Analyzer. YSI's unique enzyme technology provides for specific dextrose measurement. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

A portion of a sample is solubilized in cold water and a portion is autoclaved or chemically solubilized. The samples containing starch are treated identically with glucoamylase. The dextrose produced from this reaction is measured with the YSI 2700 SELECT. In this procedure chemical solubilization is described. See Application Note 319 for the autoclaved method. The ratio of dextrose in the cold water sample to dextrose in the chemically solubilized sample yields % cook.

When a sample is injected into the sample chamber, the dextrose diffuses into the membrane containing glucose oxidase. The dextrose is immediately oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The current flow at the electrode is directly proportional to the hydrogen peroxide concentration, and hence to the dextrose concentration.

II. Materials and Setup

A. YSI 2700 SELECT Biochemistry Analyzer - equipped with a 2365 Dextrose Membrane and 2357 Buffer.

B. Dextrose standards (2.5 g/L, 9.00 g/L). Place the 2.50 g/L solution in Cal Station #1.

C. 1N Acetate buffer.

D. Diazyme glucoamylase solution (Diazyme L-200 is available from Solvay Enzymes- Elkhart, Indiana).

E. 25% Trichloracetic Acid

F. 2N NaOH

G. 2N HCl

H. A heating unit such as a hot plate.

I. Phosphate buffer (40 g/L NaH2PO4, 10 g/L Na2HPO4 in reagent water).

J. Connect the 2700 SELECT to a suitable power source.

K. Perform the instrument and membrane check described in the Operations Manual (Section 3).

L. Volumetric glassware (Class A recommended).

M. The following instrument setup is recommended:

Sample size:	25 µL
Sample Station #	2
CalMethod	One Station

Black Probe Parameters

Chemistry	Dextrose
Unit	g/L
Calibrator	2.50 g/L
End Point	30 Sec
CalStation#	1

White Probe Parameters

Single Channel 2700	N/A
Dual Channel 2700	None

Autocal Parameters

Sample Error	ON
Temperature	1°C
Time	15 Min
Sample	5 Sam
Cal Shift	2%

III. Reagent Preparation

A. Acetate Buffer (pH 4.2) - Weigh 9.1 grams of sodium acetate into 500 mL volumetric flask. Add about 300 mLs of distilled water and mix until all the solid is dissolved. Add 22.3 mLs (23.4 grams) of glacial acetic acid. Dilute to volume with water and mix.

B. Diazyme Enzyme Solution - Pipette 30 mLs of Diazyme L-200 into a 100 mL volumetric flask. Add 0.1 gram of EDTA (Ethylenediaminetetraacetic acid) and dilute to volume with distilled water. Mix thoroughly to dissolve the EDTA and the Diazyme.

C. Hydrochloric Acid Solution (2N) - For example: Measure 82.4 mL of 36.5-38% HCl and transfer to a 500 mL volumetric flask. Let cool, dilute to volume with distilled water and mix.

D. Sodium Hydroxide Solution (2N) - For example: Weigh 40 grams of NaOH pellets into a 500 mL volumetric flask. Add 300 mL of water and mix. Let cool, dilute to volume and mix.

E. Trichloroacetic Acid Solution (25%) - Disslove 50.0 grams of TCA crystals into 200 mLs of water.

IV. Method

A. Grind sample to a fine powder.

B. Weigh out 0.50 grams of sample twice and transfer each to a 100 mL volumetric flask. Record exact weights.

C. Add 25 mLs of distilled water to each flask. Label one flask #1 and the second flask #2. To the flask labeled #1 proceed with the chemical solubilization. Set flask #2 aside until the enzymatic digestion in steps F-I.

Chemical solubilzation to determine total starch

D. Add 10 mLs 2N NaOH to the solution in flask #1. Place on a heating unit and simmer for 20 minutes. Stir gently and periodically.

E. Add 10 mLs 2N HCl following the 20 minutes and swirl the flask. Allow the flask to cool to below 50°C.

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Enzymatic digestion to determine cooked starch.

F. To both flasks (#1 and #2) add 10 mLs of 1N acetate buffer.

G. Add 5 mLs. of 30% Diazyme Enzyme Solution to each flask. Mix well and place the flask in a 40°C water bath for 70 minutes.

H. After **exactly** 70 minutes incubation, remove the flasks from the water bath. Immediately add 5 mLs of 25% TCA to each flask to stop hydrolysis.

I. Cool to room temperature and fill to volume with phosphate diluent buffer and mix well.

Blank sample

J. Since Diazyme may contain free dextrose, perform steps F-I without using the sample containing starch. Both the cold water sample and the autoclaved sample should be corrected using this value.

K. Calibrate the 2700 SELECT with a 2.50 g/L dextrose standard solution.

L. Check the linearity of the membrane at least once a day by injection of a dextrose linearity check solution (9.00 g/L). Refer to the Operators Manual (Section 3) for specifications.

M. Determination of Dextrose: Assay the blank prepared in J by aspiration into the 2700 SELECT.*

N. Determination of Cooked Starch: Assay the sample prepared in flask #2 by aspiration into the 2700 SELECT.*

O. Determination of Total Starch: Assay the sample prepared in flask #1 by aspiration into the 2700 SELECT.*

P. Calibrate frequently as described in the Operations Manual (Section 6).

* The linear range of the system is 0-9.00 g/L dextrose. If the value reported exceeds this, further dilution is required.

Note: If the sample contains free dextrose, both the cold water and the autoclaved sample will have to be corrected with this value. Weigh 0.5 grams of sample into a 100 mL volumetric flask and dilute to the mark with phosphate buffer. Mix the sample for 20 minutes and analyze.

V. Calculations

To calculate % cook, multiply the reported value by the appropriate dilution factor. The value of the blank (measured in step J) should be subtracted from the cooked starch (measured in step N) and the total starch (measured in step O).

Since 1.1 g of dextrose is produced when 1.0 g of starch is hydrolyzed, the dextrose concentration of the sample should be multiplied by 0.9.

$$\%\ \text{Cook} = \frac{[\text{cooked starch}] \times 100\%}{[\text{total starch}]}$$

or

$$\%\ \text{Cook} = \frac{[(\text{Step N} - \text{Step J}) \times 0.9] \times 100\ \%}{[(\text{Step O} - \text{Step J}) \times 0.9]}$$

Example: 0.52 g of pet food was diluted to 100 mL in a Class A volumetric flask. The sample was prepared using the enzymatic digestion procedure. When assayed, the value reported was 1.45 g/L dextrose.

A 0.52 g of pet food was diluted to 100 mL in a Class A volumetric flask. The sample was prepared using the chemical solubilization procedure. When assayed, the value reported was 1.82 g/L dextrose.

The blank contained 0.01 g/L of dextrose.

Cooked starch:

1.45 - 0.01g/L x 0.9 x 0.100 L/0.52 g = 0.249

= 24.9 %

Total starch:

1.82 - 0.01g/L x 0.9 x 0.100 L/0.52 g = 0.313

= 31.3 %

% Cook = 24.9 %/31.3% x 100% = 79.6 %

VI. Ordering Information

YSI No.	
2700	Biochemistry Analyzer
2365	Dextrose Membrane Kit
2776	Dextrose Standard Solution (2.50 g/L)
1531	Dextrose Standard Solution (9.00 g/L)
2357	Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation)