

## Determination of Cook in Extruded Cereal Products

### 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. The samples containing starch are treated identically with glucoamylase. Dextrose produced from this reaction is measured with the YSI 2700 SELECT. The ratio of dextrose in the cold water sample to dextrose in the autoclaved 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- $\delta$ -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% Trichloroacetic Acid
- F. Phosphate diluent buffer (40 g/L  $\text{NaH}_2\text{PO}_4$ , 10 g/L  $\text{Na}_2\text{HPO}_4$  in reagent water, pH 5.9).
- G. Volumetric glassware (Class A recommended).
- H. Connect the 2700 SELECT to a suitable power source.
- I. Perform the instrument and membrane check described in the Operations Manual (Section 3).
- J. The following instrument setup is recommended:

Sample size:	25 $\mu\text{L}$
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. Method

- A. Cold water sample:
  1. Into a 125 mL Erlenmeyer flask disperse ~ 0.50 g sample into ~ 43 mL reagent water. Record the exact weight of the sample.
  2. Add 5 mL of 1N Acetate buffer, pH 4.2.
  3. Add 2.5 mL of 30% Diazyme solution.
  4. Cover with aluminum foil and incubate, in water bath, for one hour at 40°C.
  5. Add ~ 3.2 mL of 25% Trichloroacetic Acid immediately after the incubation and swirl the contents.
  6. Allow the solution to cool to room temperature. Transfer the solution to a 100 mL volumetric flask and dilute with phosphate diluent buffer (pH 5.9). Shake vigorously.
- B. Autoclaved sample:
  1. Dilute the sample as in A1. Cover with aluminum foil and autoclave at 15-20 psi, ~124°C +/- 3°C for one hour. Cool to 40°C.
  2. Repeat steps A2 - A6 above.
- C. Blank sample:
 

Since Diazyme may contain free dextrose, perform steps A1 - A6 without using the sample containing starch. Both the cold water sample and the autoclaved sample should be corrected using this value.
- D. Calibrate the 2700 SELECT with a 2.50 g/L dextrose standard solution.
- E. 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.
- F. Determination of Blank: Assay the blank prepared in C by aspiration into the 2700 SELECT.\*
- G. Determination of Cooked Starch: Assay the sample prepared in A by aspiration into the 2700 SELECT.\*
- H. Determination of Total Starch: Assay the sample prepared in B by aspiration into the 2700 SELECT.\*

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

\* The linear range of the system is 0 to 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 100 mL volumetric flask and dilute to the mark with phosphate diluent buffer. Mix the sample until dissolved and analyze.

#### IV. Calculations

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

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.

$$\% \text{ Cook} = \frac{[\text{Cooked Starch}]}{[\text{Total Starch}]} \times 100 \%$$

$$\text{or } \% \text{ Cook} = \frac{[(\text{Step G}-\text{Step F}) \times 0.9]}{[(\text{Step H}-\text{Step F}) \times 0.9]} \times 100 \%$$

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

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

The blank contained 0.01 g/L of dextrose.

Cold water starch:

$$\begin{aligned} 1.45-0.01 \text{ g/L} \times 0.9 \times 0.100 \text{ L}/0.52 \text{ g} &= 0.249 \text{ g starch/g food} \\ &= 24.9 \% \text{ (w/w)} \end{aligned}$$

Total starch:

$$\begin{aligned} 1.82-0.01 \text{ g/L} \times 0.9 \times 0.100 \text{ L}/0.52 \text{ g} &= 0.313 \text{ g starch/g food} \\ &= 31.3 \% \text{ (w/w)} \end{aligned}$$

% Cook:

$$24.9 \% / 31.3 \% \times 100 \% = 79.6 \%$$

#### V. 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)

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