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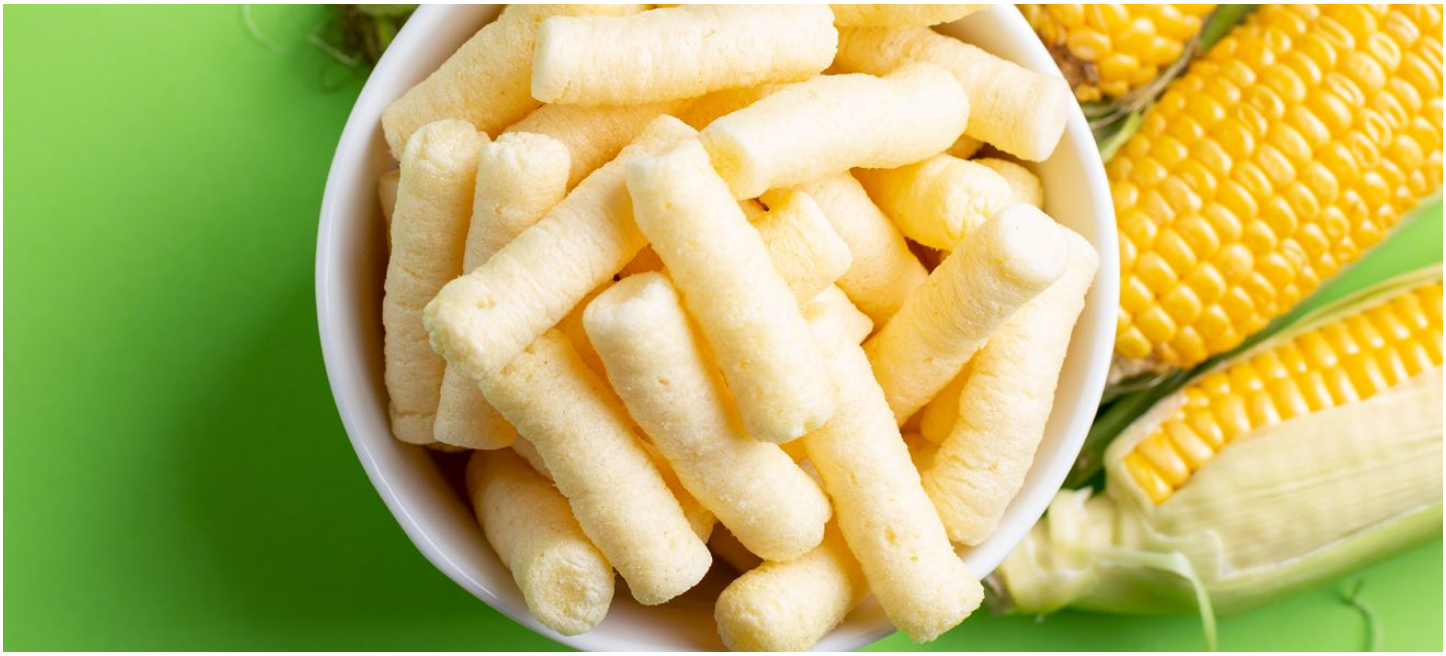
Application Note
YSI Life Sciences • 223LS-02

Food & Beverage Series

DETERMINATION OF % COOK IN EXTRUDED CEREAL PRODUCTS USING CHEMICAL SOLUBILIZATION



Introduction

The degree of cook of extruded cereal products can be determined using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for specific glucose 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 glucose produced from this reaction is measured with the YSI 2900 Series. In this procedure chemical solubilization is described. See Application Note 222LS for the autoclaved method. The ratio of glucose in the cold water sample to glucose in the chemically solubilized sample yields % cook.

When a sample is injected into the sample chamber, the glucose diffuses into the membrane containing glucose oxidase. The glucose 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 therefore is directly proportional to the glucose concentration.

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I. Materials & Setup

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2365 Glucose Membrane and 2357 Buffer.

B. Glucose standards (2.5 g/L, 9.00 g/L).

C. IN Acetate buffer

D. Glucoamylase solution

E. 25% Trichloracetic Acid

F. 2N Sodium Hydroxide

G. 2N Hydrochloric Acid

H. A heating unit such as a hot plate power source.

I. Phosphate buffer (40 g/L NaH_2PO_4, 10 g/L Na_2HPO_4 in reagent water).

J. Connect the 2900 Series instrument to a suitable power source.

K. Perform the instrument and membrane daily checks described in the Operations Manual.

L. Volumetric glassware (Class A recommended).

M. The following instrument setup is recommended: Sample Size 25 µL

Probe A Parameters		**Autocal Parameters**	
Chemistry	Glucose	Temperature	1°C
Unit	g/L	Time	30 Min
Calibrator	2.50	Sample	5 Sam
End Point	30 Sec	Cal Shift	2%

II. Reagent Preparation

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

B. Glucoamylase Enzyme Solution - Pipette 30 mL of glucoamylase 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 glucoamylase.

C. Hydrochloric Acid Solution (2N) - For example: Measure 82.4 mL of 36.5-38% hydrochloric 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 sodium hydroxide pellets into a 500 mL volumetric flask. Add 300 mL of distilled water and mix. Let cool, dilute to volume and mix.

E. Trichloroacetic Acid Solution (25%) - Dissolve 50.0 grams of TCA crystals into 200 mL of distilled water.

III. 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 mL 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 solubilization to determine total starch

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

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

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% glucoamylase 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 mL 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 glucoamylase may contain free glucose, 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 2900 Series instrument with a 2.50 g/L glucose standard solution.

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

M. Determination of Glucose: Assay the blank prepared in J by aspiration into the 2900 Series instrument.

N. Determination of Cooked Starch: Assay the sample prepared in flask #2 by aspiration into the 2900 Series instrument.

O. Determination of Total Starch: Assay the sample prepared in flask #1 by aspiration into the 2900 Series instrument.

P. Calibrate frequently as described in the Operations Manual.

Note: If the sample contains free glucose, 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.

Calculations

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

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

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 glucose.

The blank contained 0.01 g/L of glucose.

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

or

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

Cooked starch: 1.45 - 0.01 g/L x 0.9 x 0.100L/0.52 g	= 0.249 = 24.9 %
Total starch: 1.82 - 0.01 g/L x 0.9 x 0.100L/0.52 g	= 0.313 = 31.3%
% Cook = 0.249 / 0.313 x 100%	= 79.6%

Potassium Ferrocyanide

Glucose Standard Solution (9.00 g/L)

Glucose Standard Solution (2.5 g/L)

NaCl Solution

Buffer Kit

Glucose Membrane Kit

YSI 2900 Biochemical Analyzer

Ordering Information

2900	Biochemistry Analyzer
2365	Glucose Membrane Kit
2776	Glucose Standard Solution (2.50 g/L)
1531	Glucose Standard Solution (9.00 g/L)
2357	Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation)

The YSI 2900 Series Biochemistry Analyzers offer a wide range of configurations, options and accessories to meet the needs of various industry applications.

YSI Life Sciences develops and manufactures scientific instruments, sensors and systems that serve a variety of scientific and industrial markets worldwide. YSI has a long history in the life sciences and bioanalytical markets, most notably with our introduction of the world's first commercial whole blood glucose analyzer in 1975. Today there are over 10,000 YSI instruments installed around the world, trusted in critical situations to provide the most accurate data in the shortest time.

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