Food & Beverage

ANALYTICAL METHODS MANUAL

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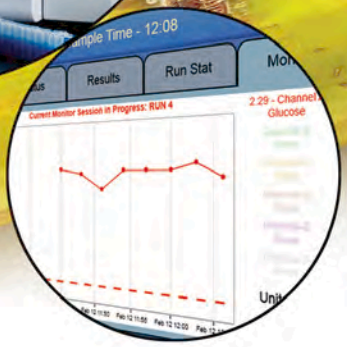
THERE'S A **SCIENCE** TO FLAVOR



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Work easier with our intuitive, graphical user interface and touch screen display.
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Food Analysis
SIMPLIFIED

William Miller
YSI Life Sciences Product Manager

There is a science to flavor. The field of food science has evolved significantly to meet the growing demands for food composition and characteristic determination. Trends and demands of consumers, the food industry, and national and international regulations challenge food scientists as they work to monitor food composition, authentication and to ensure the quality and safety of the food supply[1].

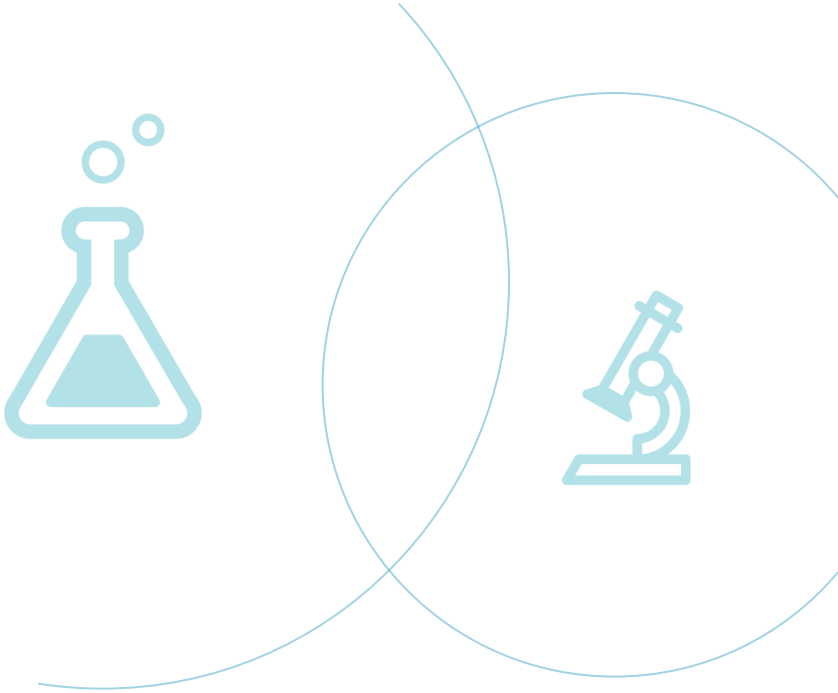
For example, regulatory compliance with the US Food & Drug Administration's (FDA) Nutrition Labeling and Education Act and other country-specific regulatory requirements, mandate disclosure and labelling of food ingredients and their respective quantities. Also, more consumers are interested in food product nutrient content, so that they may make informed purchase decisions based on diet and health.

Lastly, product quality management and competitive pricing are driving industry trends in analytical methods and technologies. Analytical methods must be applied across the entire food supply chain to achieve the desired final product quality[1]. Cost reduction initiatives and workforce downsizing mandate the implementation of economical, easy-to-use technologies. Thus, simple, costeffective, standardized technology platforms, providing rapid, accurate results, are of the essence for today's food and beverage space.

Achieving these industry requirements can be a challenge. However, YSI Life Sciences' family of Biochemistry Analyzers can meet these demands through automated, plug-and-play analytical solutions for raw materials testing, food R&D, in-process monitoring and final product testing – especially when rapid, precise and economical analysis of saccharides, amino/organic acids, alcohols and electrolytes are required.

YSI Biosensor Technology Advantage
YSI's innovative enzyme electrode technology harnesses the power of enzymatic specificity and catalysis to provide a rapid, precise analytical tool. Enzymes, which are powerful biological catalysts, can accelerate reactions by factors of at least one million[2]. Additionally, enzymes are highly specific for both the reaction catalyzed as well as its choice of substrate[2]. YSI immobilizes substrate-specific enzymes between two selective interfering membranes and couples the membrane component to a platinum electrode, which results in a highly specific measurement for a given substrate.

YSI Life Sciences' family of Biochemistry Analyzers can meet demands through automated, plug-and-play analytical solutions for raw materials testing, food R &D, in-process monitoring and final product testing – especially when rapid, precise and economical analysis of saccharides, amino/organic acids, alcohols and electrolytes are required.



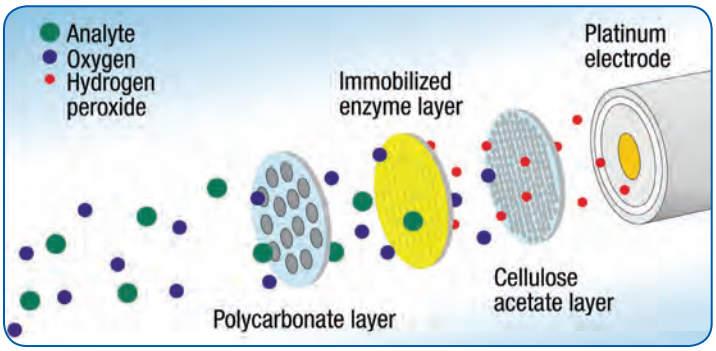


Figure 1
YSI Enzyme Electrode Technology. Enzyme specificity and proprietary membrane attributes allow for rapid, precise and essentially interference-free analysis.

Figure 2
The YSI 2950 Biochemistry Analyzer can simultaneously measure up to six chemistries for raw materials, R&D, in-process and final product sample analysis.

To better explain how the enzyme electrode technology works, an example of a common food ingredient analysis, i.e., dextrose (D-glucose), will be used. An enzyme probe is fitted with a three-layer membrane containing immobilized glucose oxidase enzyme in the middle layer. The face of the probe, covered by the membrane, is situated in a buffer-filled sample module into which the dextrose sample is injected by the instrument. The dextrose diffuses through the first membrane. When it contacts the immobilized glucose oxidase, it is rapidly oxidized, producing hydrogen peroxide (H_2O_2).

H_2O_2, in turn, passes through the inner membrane and is oxidized at the platinum anode, producing electrons. A dynamic equilibrium is achieved when the rate of H_2O_2 production and the rate at which H_2O_2 leaves the immobilized enzyme layer are constant and is indicated by a steady state response. The electron flow is linearly proportional to the steady state H_2O_2 concentration and, therefore, to the concentration of the substrate. All of this occurs in a matter of seconds, which results in a sample analysis cycle of 60 seconds or less per chemistry analyzed. See Figure 1.

Sample analysis requires little or no preparation due to the YSI Biochemistry Analyzer's unique sample chamber design and membrane characteristics, which makes the enzyme electrode impervious to sample color, pH, turbidity, cell concentrations, salts, proteins, detergents and other low molecular weight interferences. Additionally, only small sample volumes are needed, ranging between 10–50 µl. The YSI 2900 Series Biochemistry Analyzers are highly flexible, modular platforms with a range of configurations, options and accessories to meet the various needs of the food process cycle, regardless of scale of operations (Figure 2).

Up to six different chemistries can be measured simultaneously using automated, high-throughput options, i.e.,

96-well plates, stat mode for immediate process sample checks and automated on-line sample analysis for the food and beverage process and effluent streams. Each analyzer offers an intuitive graphic user interface, onboard training videos and multiple data management options. As compared to other conventional methods, such as HPLC, YSI Biochemistry Analyzers offer a low-cost alternative for both initial capital investment and cost per chemistry test ($0.10 - $0.70 USD/sample). Other features include low maintenance requirements and easy product changeover.

Industry Applications

For over two decades YSI's Biochemistry Analyzers have been the analytical technology of choice for many major food and beverage manufacturers. The operations in which the YSI biosensor technology has been utilized spans from raw materials analysis through final product QC testing and effluent monitoring. The table below lists some of the food and beverage applications in which YSI Biochemistry Analyzers have been employed.

Table 1

Examples of YSI End-User Current and Emerging Applications

Analytical Application	Function
Choline in infant formula	R&D, In-process
Choline in animal feeds	Final product
Dextrose, Sucrose & Lactose in candy	Final product
Dextrose & Sucrose in cereal	R&D, In-process
Dextrose - starch-to-glucose conversion	R&D, In-process
Dextrose & Sucrose in potatoes/french fries	Raw materials, In-process
Dextrose & Lactate in wine production	In-process
Glutamate (MSG) in broth & food bases	Final product
Lactose in cheese filtration	R&D
Lactose in low lactose milk product	In-process
Lactate in tomato-based products	In-process, Final product
Sucrose content in soft drinks	In-process

Conventional methods can be costly and/or time consuming. The YSI biosensor technology offers a low cost alternative while providing rapid sample analysis due to the characteristics of the immobilized enzyme membrane technology. Additional time savings can be realized as no sample preparation is required in most cases. For example, analyzing glucose and sucrose in high salt food samples is easily achieved with no sample preparation. In contrast, trying to perform this type of analysis using HPLC is difficult, and sometimes impossible, due to high salt interference. Other food and beverage applications in which the YSI enzyme electrode technology has replaced other analytical methods include various analyte monitoring of ice cream products, beer production, sugar-free food and beverages, dry bakery mixes and animal food.

As most of this discussion has implied at-line or off-line laboratory use, YSI Biochemistry Analyzers have been successfully employed for on-line process monitoring. With industry trends moving towards automated, online and inline monitoring of food and beverage processes, real-time monitoring of food ingredients and effluent can be achieved with YSI's online monitoring and control system. This bolt-on function automatically draws a fluid sample from the process stream and delivers the sample directly to the YSI Biochemistry Analyzer. The sampling is performed aseptically, and the online analytical results can be communicated immediately to your SCADA or process management system for real-time data acquisition and/or feedback control.

In conclusion, food industry trends, regulatory requirements and needed analytical improvements dictate the requirement for simplified, low-cost, rapid and precise analysis of food and beverage ingredients throughout the entire process train. These attributes should be coupled with versatile, scale-independent technologies to provide standardized, plug-and-play analytical platforms which span across the food and beverage cycle.

YSI's unique enzyme biosensor technology helps satisfy this need for common food ingredients including mono- and disaccharides, alcohols, amino acids and more. If you would like to learn more about YSI's food and beverage analytical solutions, please contact us at ysi.com/lifesciences, info@ysi.com or 800-765-4974. ■

References

[1] S. Suzanne Nielsen, ed. Food Analysis. New York: Springer, 2010.

[2] Stryer, Lubert. Biochemistry. New York: W.H Freeman and Company, 2000.

Simultaneous Measurement of L-Lactate and Ethanol in Tomato-Based Products

INTRODUCTION

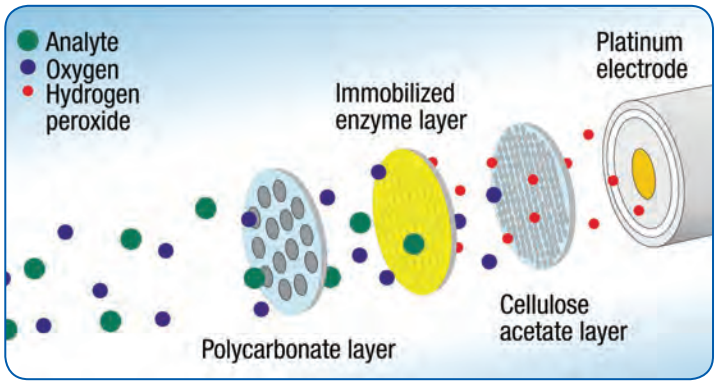
L-lactate and ethanol concentrations in complex matrices such as tomato paste and ketchup can be measured in about one minute with minimal preparation using a YSI Biochemistry Analyzer. YSI's unique enzyme electrode technology provides for specific lactate measurements in the range of 10 to 1335 ppm (mg/L). Ethanol can be measured in the range of 20 to 1000 ppm. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of chemical substances.

When configured with the YSI 2329 lactate oxidase membrane, a YSI 2786 alcohol oxidase membrane and YSI 1579 buffer, the YSI analyzer simultaneously measures lactate and ethanol after aspiration of just 15 microliters of sample. Samples may require centrifugation to remove particulates; therefore the lactate and ethanol are read from the supernatant. Lactate concentrations that exceed 1335 ppm and ethanol concentrations that exceed 1000 ppm may require dilution. Results are displayed and printed. The sample automatically flushes from the electrode chamber 45 seconds after results and the YSI analyzer is ready to measure the next sample. Turn around time is under two minutes.

In the manufacture and packaging of ketchup and related tomato products, both lactate and ethanol concentrations can be used to quantify microbial testing of ingredients, in-process and finished products. Lactate-producing bacteria and ethanol-producing yeast may both contribute to microbial load. When mixed with broth and grown in cultures for 1-5 days, the lactate and ethanol concentrations may be correlated with the degree of microbial load. This method of measuring lactate and/or ethanol typically reduces test time by hours and provides results that better predict potential flavor issues and incipient spoilage compared to traditional microbial methods.[1]

Data in this study supports the feasibility of simultaneously measuring L-lactate and ethanol in tomato-based products. Lactate and ethanol were measured in supernatants of a diluted ketchup sample that was obtained from a commercially available source. In order to prove feasibility ketchup samples were spiked with lactate and ethanol to levels that would be typical of changes seen in microbial load tests (50 to 150 ppm range for both lactate and ethanol).

Precision of replicate samples was determined from selected samples; and percent recovery was determined for samples spiked with both lactate and ethanol. The material and method sections are written to demonstrate one approach to measuring lactate and ethanol in a tomato process application. The results section demonstrates typical precision and accuracy when using a YSI Biochemistry Analyzer in process applications.



$$\text{Analyte} + O_2 \xrightarrow{\text{Enzymatic oxidase}} H_2O_2 + \text{By product}$$

$$H_2O_2 \xrightarrow[+0.7V]{\text{Oxidized at probe}} O_2 + 2H^+ + 2e^-$$

Substrate proportional to electron flow...

$$\text{Substrate} \longrightarrow 2e^-$$

YSI 2900

YSI 2950

METHODS

A sample from commercially available ketchup was collected and diluted 1:1 by volume with reagent water to reduce viscosity. Two aliquots of the sample were collected and transferred to 1.5 ml plastic 'eppy' tubes, and then spun by centrifuge. The supernatants were presented to the YSI Biochemistry Analyzer for ten (10) measurements each of L-lactate and ethanol. The readings were recorded and the precision of each analyte was determined. The final base lactate and ethanol readings were averaged and used to calculate spike/recovery values in the second study.

Since levels of lactate as low as 50 ppm (mg/L) can indicate potential flavor/spoilage issues in tomato products during microbial load tests as determined by human taste testers (personal communication, HJ Heinz), and changes in lactate levels in cultures of approximately 100 ppm can indicate incipient spoilage, the spike/recovery tests were designed with this in mind. Ethanol levels have been less studied, however changes in the 50 to 150 ppm range represents a reasonable change to detect yeast or mold effects in the microbial load tests.

YSI 1530 (30 mmol/L; 267 mg/dL L-lactate) was used as a stock lactate standard. YSI 2790 (3.20 g/L; 320 mg/dL ethanol) was used as a stock ethanol standard. Both were prepared by YSI metrologists with metrology-grade glassware and weights using the highest purity standards available.

Into a 100 ml volumetric cylinder 2.000 ml of lactate standard and 2.000 ml of ethanol standard were combined with 96 ml of ketchup that had been diluted 1:1 with reagent water. The additions represent 53.4 mg of lactate and 64.0 mg of ethanol. These values represent theoretical changes of 53.4 ppm and 64.0 ppm increases above base lactate and ethanol concentrations, respectively after corrected for volume (0.96 x unspiked conc.).

Following protocol described in the previous paragraph, 4.000 ml of lactate and 4.000 ml of ethanol were combined with 92 ml of diluted ketchup. The theoretical changes were determined to be 106.8 ppm and 128.0 ppm above base for lactate and ethanol, respectively.

RESULTS

YSI Biochemistry Analyzer Precision for Unspiked Samples

Selected samples of diluted ketchup (1:1) were used for precision studies. Ten (10) replicates of each sample were performed. Results are shown in tables on page 6 for lactate and for ethanol.

The standard deviation (STD) was determined for each replicate series. Imprecision was no greater than 2.25% expressed as CV.

Percent Recovery of Spiked Samples

As an evaluation of measurement accuracy, spiked samples were measured using the YSI Biochemistry Analyzer. The spiked sample values were then compared to the calculated spiked sample value. To clarify the process, a sample of known concentration, referred to as the unspiked sample, is combined with a spike of known concentration to create the spiked sample. The expected value of the spiked sample is listed in the table (pg 6) as the "Calculated" value. The value of the spiked sample obtained with the YSI Biochemistry Analyzer is then designated the "Spiked" value.

Spiked samples of diluted ketchup were measured using the YSI Biochemistry Analyzer within 30 minutes of spiking and mixing. Aliquots were each measured in triplicate and the average result was recorded for each. The results are shown in Table 1 on page 6.

SUMMARY

Experimental results shown above demonstrate that the YSI Biochemistry Analyzer can simultaneously measure lactate and ethanol in a tomato matrix with adequate precision and accuracy to make process and quality assurance decisions in tomato product manufacturing. Although the methods section describes a protocol appropriate for screening potential flavor issues and incipient spoilage in finished product, similar approaches provide means to test microbial load in ingredients or to assess sanitation needs in process equipment. L-lactate is useful in identifying growth of lactic acid bacteria while ethanol identifies the potential presence of yeasts and molds.

L-LACTATE

Sample	Replicates	Mean ppm	STD ppm	CV (%)
LAC-B1	10	72.5	1.28	1.76%
LAC-B2	10	72.8	1.64	2.25%

ETHANOL

Sample	Replicates	Mean ppm	STD ppm	CV (%)
ETH-B1	10	181.7	3.97	2.19%
ETH-B2	10	181.2	3.85	2.13%

Table 1

Sample	Spike*	Unspiked*	Spiked*	Calcu-lated*	Recovery
LAC-1	53.4	72.6	122.3	123.1	99.4%
LAC-2	106.8	72.6	175.3	173.6	101.0%
ETH-1	64.0	181.5	244.8	238.2	102.8%
ETH-2	128.0	181.5	307.2	295.0	104.1%

*All values in ppm or mg/L units

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2328 Lactate Linearity Test Standard (15.0mmol/L)
2329 L-Lactate Oxidase Membrane Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation)
2776 Glucose/Lactate Calibrator (0.50 g/L lactate)
2786 Alcohol Oxidase Membrane Kit
1579 Buffer Kit
2792 Low Concentration Ethanol Kit (0.50 g/L; 1.00 g/L)
- 2759 0.50 g/L Ethanol Standard
- 2769 1.0 g/L Ethanol Standard ■

[1]YSI greatly appreciates the contributions of John Palombi, Laura Bautista and Phil Vendemio (all of Heinz North America) who provided valuable insight and understanding regarding quality control in tomato product processing.

Ethanol Determination in Beer

INTRODUCTION

Ethanol concentrations in complex matrices such as beer can be measured directly and quickly using any YSI 2900 Series Biochemistry Analyzer. YSI's unique biosensor technology provides for rapid ethanol measurement. Measurements are virtually unaffected by color, turbidity, density, or pH.

When a sample is injected into the sample chamber, the ethanol diffuses into the membrane containing alcohol oxidase. The ethanol is immediately oxidized to hydrogen peroxide and acetaldehyde. 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 ethanol concentration.

Other alcohols can interfere with ethanol measurement by also oxidizing and producing a signal. Fortunately, most alcohols have much lower responses to alcohol oxidase than ethanol. One notable exception is methanol, which is over three times as sensitive to the enzyme as ethanol. Therefore, samples must be methanol-free.

This application note demonstrates how simply and quickly ethanol concentrations can be determined using the following method. Precision of replicate samples was determined from selected samples; and percent recovery was determined for samples spiked with ethanol. The results section demonstrates typical precision and accuracy when using a YSI Biochemistry Analyzer in process applications.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2786 Ethanol Membrane and 1579 Ethanol Buffer.

B. 2790 Ethanol standards (2.00 g/L, 3.20 g/L). Place the 2.00 g/L calibrator solution in Station 2.

C. Connect the 2900 Series Biochemistry Analyzer to a suitable power source.

D. Perform the instrument and membrane check described in the Operations Manual.

E. Volumetric glassware (Class A recommended).

YSI 2900

F. The following instrument setup is recommended:

Probe A Parameters

Chemistry	Ethanol
Unit	g/L
Calibrator	2.00 g/L
Sample size	15 µL
End Point	45 Sec
Cal Station	2[1]

Probe B Parameters

B Chemistry	None

Autocal Parameters

Time (min)	30
Sample	5
T Shift (°C)	1
Cal Shift (%)	2

[1] Calibrator should be used in small quantities and refreshed frequently due to evaporation. Calibration should be performed from a test tube.

II. METHOD

A. Dilute sample to bring ethanol concentration into the linear range of the instrument, which is 0.04 to 3.20 g/L. Samples with 1.5 to 2.5 g/L ethanol will give the best results.

B. Calibrate the 2900 Series Biochemistry Analyzer with a 2.00 g/L ethanol standard solution.

C. Check the linearity of the membrane at least once a day with an ethanol linearity check solution (3.20 g/L). Refer to the User's Manual for specifications.

D. Assay the sample prepared in A by aspiration into the 2900 Series Biochemistry Analyzer. The linear range of the system is 0.04 to 3.20 g/L ethanol. If the value reported exceeds this, further dilution is required.

E. Calibrate frequently as described in the User's Manual.

III. CALCULATIONS

To calculate % ethanol, multiply the reported value by the appropriate dilution factor.

Example: 5.00 mL of beer was diluted to 100 mL in a Class A volumetric flask. When assayed, the value reported was 1.56 g/L ethanol.

% Ethanol: 1.56 g/L x 0.100L/5mL	= 0.03120g ethanol/mL beer
	= 3.12% (w/v)

For a v/v result, divide the mass by the density of ethanol (0.789 g/mL at 20°C).

0.03120 g/mL / 0.789 g/mL	= 0.0395mL ethanol/mL beer
	= 3.95% (v/v)

YSI 2900 Biochemistry Analyzer

IV. RESULTS / DISCUSSION

Several beers were analyzed for their ethanol concentrations (w/v) using the YSI biochemistry analyzer and Sigma Test Kit 332-BT. The YSI analyzer results and Sigma Test Kit results are compared in the following table:

	YSI	Sigma
Beer A	4.40%	4.39%
Beer B	3.75	3.76
Beer C	3.89	3.87
Beer D	3.74	3.79

YSI's proprietary immobilized enzyme membrane technology provided accurate ethanol results within one minute of sampling. The YSI 2900 Biochemistry Analyzer's ability to provide rapid, precise analyses makes it ideal for brewing process monitoring and control and QC analysis of beer harvest samples.

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2786 Ethanol Membrane Kit
2790 Ethanol Standards Kit
1579 Carbonate Buffer Concentrate
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

J. Lohr Winery Utilizes YSI Instruments in Managing Dissolved Oxygen

INTRODUCTION

Although oxygen is a necessary component in the wine aging process, it can also be detrimental to overall wine quality. Too much oxygen can cause browning in white wines and induce flavor degradation in both red and white wines. The J. Lohr Winery in San Jose, California, consistently strives to minimize and control oxygen exposure to their premium wines throughout their winemaking process.

DISSOLVED OXYGEN (DO) DETERMINATION

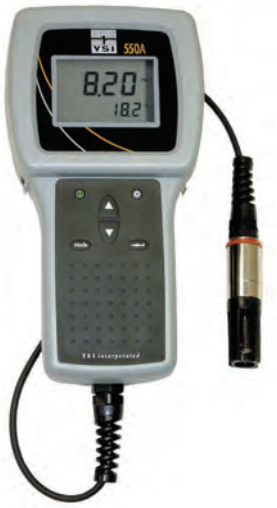
Using the YSI 550A handheld DO and temperature instrument, the laboratory staff at J. Lohr Winery can quickly and simply check DO levels of wine stored in stainless steel tanks and oak barrels. Obtaining DO concentrations in these vessels provides a baseline measurement to determine the amount of oxygen introduction of the bottling process when the wine is moved from one location to another through pumps and hoses. Fittings on tank valves, hoses, and pumps can be checked as potential sources of the oxygen introduction. Preventative measures, such as nitrogen gas sparging can greatly diminish the amount of oxygen introduction during wine transfer.

Oxygen monitoring after bottling ensures minimal oxygen introduction from the bottling tank to a 54-valve filler. The YSI 5100 Dissolved Oxygen Instrument is used to determine the dissolved oxygen levels and temperature of the wine once it is bottled. These measurements are achieved using the YSI 5010-W, Wine Bottle BOD Probe, which is specifically designed to fit directly into the neck of a wine bottle. The 5010-W probe has a tapered fit, which allows a best fit and seal atop the bottle. The probe also has a self-powered stirrer attached to its submersible end, which ensures an optimal flow of wine past the membrane within the small confines of the bottle.

WINE PROCESS MONITORING

DO levels can be checked at various points in the wine bottling process, such as filler bowl seals and filler spouts. DO baseline values are determined from the bottling tank using the 550A handheld DO instrument.

Bottles are then pulled from the bottling line after the corker and checked immediately in the laboratory using the 5100 and 5010-W wine bottle BOD probe. The results are used for wine Quality Assurance/Quality Control. If it is determined that too much oxygen has been introduced into the wine, corrective measures can be quickly implemented to rectify the problem. This type of careful inspection allows J. Lohr Winery to consistently produce high-quality wines.



YSI 550A Dissolved Oxygen instrument

Today the J. Lohr products are available throughout the United States and in more than 25 countries worldwide. The goal of J. Lohr Winery is to produce the finest varietals in the world, using a style that focuses on flavor and complexity through vineyard selection, technology and innovation.

This goal has led Jerry Lohr, President and Owner, and his team to develop three tiers of wines produced from estate vineyards: J. Lohr Cuvée Series, J. Lohr Vineyard Series, and J. Lohr Estates. In addition, J. Lohr Winery produces three tiers of wines to meet the needs of everyday and entry-level wine consumption: Crosspoint Vineyards, Cypress, and Painter Bridge. ■

For additional information regarding the J. Lohr Wine please visit: jlohr.com

For additional information regarding the measuremer DO at the J. Lohr Winery, please contact:
Susan Kanzaki
Lab Supervisor, J. Lohr Win

YSI 5100 and 5010-W measure dissolved oxygen directly in the wine bottle.

Choline Determination

INTRODUCTION

Choline concentrations in complex matrices can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for specific choline measurement. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a sample is injected into the sample chamber, the choline diffuses into the membrane containing choline oxidase. The choline is immediately oxidized to hydrogen peroxide and betaine. 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 choline concentration.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2771 Choline Membrane and 2357 Buffer.

B. Choline standards (175 mg/L, 450 mg/L).

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

D. Perform the instrument and membrane daily checks described in the User's Manual.

E. Volumetric glassware (Class A recommended).

F. The following instrument setup is recommended. Sample size: 25 µL

Probe A Parameters

Chemistry	Choline
Unit	mg/L (ppm)
Calibrator	175 mg/L
End Point	30 Sec

Probe B Parameters

B Chemistry	None

Autocal Parameters

Time (min)	30
Sample	2
T Shift (°C)	1
Cal Shift (%)	2

II. METHOD

A. Total choline concentration should not exceed 450 mg/L, as determined on Part D below; otherwise the sample will require further dilution. Use volumetric glassware for all dilutions. Dilute with either water or 2357 buffer.

B. Calibrate the 2900 series instrument with a 175 mg/L Calibration Standard.

C. Check the linearity of the membrane at least once a day by injection of a choline linearity check solution (450 mg/L). Refer to the User's Manual (Section 5) for specifications.

D. Assay the sample by aspiration into the 2900 series instrument. The linear range of the system is 5 to 450 mg/L choline. If the value reported exceeds this, further dilution is required.

E. Calibrate frequently as described in the User's Manual.

III. CALCULATIONS

To calculate % choline, multiply the reported value by the appropriate dilution factor.

Example: 5.0 grams of pet food and 100 mL of water were mixed in a blender for 5 minutes. The supernatant was analyzed for choline. The value reported was 77.0 mg/L choline.

% Choline: 77.0mg/L x 0.100 L/5000 mg	= 0.0015 g choline/g pet food
	= 0.15% (w/w)

Example: Infant formula was aspirated into the 2900 Series (no dilution). The choline content was as follows:

Sample	mg/L Choline
Infant Formula A	232
Infant Formula B	138
Medical Nutritional Formula A	398
Medical Nutritional Formula B	386

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2771 Choline Membrane Kit
2772 Choline Standard Solution (175 mg/L)
2773 Choline Standard Solution (450 mg/L)
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

Simultaneous Measurement of Glucose and Sucrose Utilizing External Hydrolysis

INTRODUCTION

Dextrose (D-glucose) and sucrose concentrations in complex matrices can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid glucose and sucrose measurement. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a YSI 2900 Series Biochemistry Analyzer is equipped with a glucose membrane, both sucrose and glucose concentrations can be measured. This is accomplished by first determining the glucose concentration. The sucrose is then converted to glucose, and the total glucose concentration is measured. The difference in the responses corresponds to the sucrose concentration.

After a sample is injected into the sample chamber, the glucose diffuses to the glucose membrane, which contains glucose oxidase, and is oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The current produced is directly proportional to the hydrogen peroxide and glucose concentrations.

For more information on this system, refer to the Operations Manual.

I. MATERIALS & SETUP

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

B. Glucose (1.80 g/L) standard solution.

C. Buffer Diluent (40 g/L NaH_2PO_4, 10 g/L Na_2HPO_4 in reagent water).

D. Invertase - Sigma Chemical Company I-4504 recommended.

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

F. Perform the instrument and membrane check described in the Operations Manual.

G. Volumetric glassware (Class A recommended).

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

Probe A Parameters

Chemistry	Glucose
Unit	g/L
Calibrator	1.80 g/L
End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

In this section two different case studies will be described. These examples can be followed when doing analysis on products that may contain similar concentrations of glucose and sucrose.

Case #1

Example: The product is a powdered seasoning mix that is believed to contain 14% glucose and 2% sucrose. The following sample preparation was used:

A. Weigh out about 10 grams of the powder (record exact weight).

B. Transfer the powder to a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about 20 minutes.

C. Remove about 3 mL of the solution in B and add ~2 mg of invertase enzyme. Stir gently until dissolved. Cover the sample and allow to incubate at room temperature for 20 minutes.

D. Calibrate the 2900 Series with 1.80 g/L glucose standard solution.

E. Check the linearity of the membrane at least once a day by injection of an appropriate linearity standard. Refer to the Operations Manual for specifications.

F. Assay the sample prepared in B by aspiration into the 2900 Series instrument. This is the free glucose concentration (D_{free}).

G. Assay the sample prepared in C (with invertase). The value reported is the sum of the free glucose and that produced from sucrose hydrolysis (D_{total}).

H. Calibrate frequently as described in the Operations Manual.

Case #2

The product is a hard candy that is believed to contain 13% sucrose and 12% glucose. With this sample two separate dilutions are necessary. This is due to the glucose membrane reading both free glucose and the glucose produced from the hydrolysis of sucrose. The sum of these two concentrations exceeds the linear range. When analyzing the sample treated with invertase a more dilute sample will be needed. The following sample preparation was used:

A. Grind the sample into a fine powder.

B. Transfer 10 grams (record exact weight) of sample, from step A into a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about 20 minutes.

C. Transfer 5 grams (record exact weight) of sample, from step A into a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix.

D. Remove about 3 mL of the solution from C and add ~2 mg of invertase enzyme. Stir gently until dissolved. Cover the sample and allow to incubate at room temperature for about 20 minutes.

E. Calibrate the 2900 Series with a 1.80 g/L glucose standard solution.

F. Check the linearity of the membrane at least once a day by injection of an appropriate linearity standard. Refer to the Operations Manual for specifications.

G. Assay the sample prepared in B by aspiration into the 2900 Series instrument. This is the free glucose concentration (D_{free}).

H. Assay the sample prepared in C (with invertase). The value reported is the sum of the free glucose and that produced from sucrose hydrolysis (D_{total}).

I. Calibrate frequently as described in the Operations Manual.

III. CALCULATIONS

Case #1

To calculate % glucose, multiply the reported value (D_{free}) by the appropriate dilution factor.

Example: A 10.10 g powdered seasoning mix sample was prepared as described in II. B and C. When assayed, the value reported (D_{free}) was 14.6 g/L glucose.

% Glucose: 14.6 g/L x 0.100 L/10.10 g	= 0.1446 gram glucose/gram powdered mix = 14.5% (w/w)

To calculate % sucrose, subtract D_{free} from D_{total} and multiply by the appropriate dilution and mass ratio factors.

When the sample containing invertase was assayed, the value reported was 15.8 g/L (D_{total}) glucose.

$$\text{\% Sucrose: } (15.8 \text{ g/L} - 14.6 \text{ g/L}) \times \frac{0.100 \text{ L}}{10.10 \text{g}} \times \frac{342.30 \text{ g/L sucrose}}{180.16 \text{g/L glucose}}$$

= 0.0226 gram sucrose/gram powdered mix

= 2.26% (w/w)

Case #2
To calculate % glucose, multiply the reported value (D_{free}) by the appropriate dilution factor.

Example: A 10.10 g ground hard candy sample was prepared as described in II B. When assayed the value reported (D_{free}) was 12.3 g/L glucose.

% Glucose:
(12.3 g/L x 0.100 L /10.10 g = 0.1218 gram glucose/ gram candy
= 12.2% (w/w)

To calculate % sucrose, multiply the reported value (D_{total}) by the appropriate dilution factor. Then subtract D_{free} from D_{total} and multiply by the mass ratio factor.

Example: A 5.05 g ground hard candy sample was prepared as described in II D. When assayed the value reported (D_{total}) was 13.5 g/L.

% Sucrose:
(13.5 g/L x 0.100 L/5.05 g = 0.2673 gram glucose/ gram candy
= 26.7%
26.7% - 12.2% = 14.5%

$$14.5\% \times \frac{342.30 \text{ g/mole sucrose}}{180.16 \text{g/mole glucose}}$$

= 27.5% (w/w) sucrose

ORDERING INFORMATION

YSI Part Numbers:

2900	Biochemistry Analyzer
2365	Glucose Membrane Kit
2747	Glucose Standard Solution (1.80g/L)
1531	Glucose Standard Solution (9.0g/L)
2357	Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation) ■

Simultaneous Measurement of Glucose and Sucrose in Peanut Butter

INTRODUCTION

Dextrose (D-glucose) and sucrose concentrations in complex matrices such as peanut butter can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid glucose and sucrose measurements. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a 2900 Series Biochemistry Analyzer is equipped with a glucose and a sucrose membrane, simultaneous measurement of both analytes is possible. Because glucose interferes with sucrose analysis, it is necessary to follow this protocol when analyzing for sucrose in the presence of glucose.

When a sample is injected into the sample chamber, the sucrose diffuses to the sucrose membrane, which contains invertase, mutarotase, and glucose oxidase. The sucrose is hydrolyzed to α-D-glucose and fructose. The mutarotase allows for the quick equilibrium of glucose between its α and β forms. In the presence of glucose oxidase, the β-D-glucose (glucose) is oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The glucose in the sample diffuses to both the glucose and sucrose membranes, which contain glucose oxidase, and is oxidized as well. Subtracting the response of the glucose membrane from the response of the sucrose membrane yields the response due to sucrose alone. The glucose response is taken directly from the glucose membrane. The algorithm in the instrument software calculates the net concentrations. For more information on this system, refer to the Operations Manual.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2703 Sucrose Membrane, a 2365 Glucose Membrane and 2357 Buffer.

B. Glucose (2.50 g/L, 25.00 g/L) and Sucrose (5.00 g/L, 25.0 g/L) standard solutions.

C. Buffer Diluent (40 g/L NaH_2PO_4, 10g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

G. The following instrument setup is recommended:

Sample size: 10 µL

Probe A Parameters

Chemistry	Glucose
Unit	g/L
Calibrator	2.50 g/L
End Point	30 Sec

Probe B Parameters

Chemistry	Glucose
Unit	g/L
Calibrator	5.00 g/L
End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

A. Weigh between 5.00 and 10.0 grams of peanut butter to be analyzed

B. Transfer the sample to a beaker and add about 50 mL of buffer. Allow the sample to stir until the peanut butter is in solution

C. Transfer the sample to a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about twenty minutes before analysis.

D. Calibrate the 2900 Series instrument with 2.50 g/L glucose and 5.00 g/L sucrose standard solutions.

E. Check the linearity of the membrane at least once a day by injection of glucose (25.0 g/L) and sucrose (25.0 g/L) linearity check solutions. Refer to the Operations Manual for specifications.

F. Assay the sample prepared in C by aspiration into the 2900 Series instrument.*

G. Calibrate frequently as described in the Operations Manual.

* The linear range of the system is 0 to 25.0 g/L for both glucose and sucrose. The combined concentration of glucose + sucrose cannot exceed 25 g/L. If the sum of the values reported exceeds this, further dilution of the sample is required. If the glucose concentration exceeds the sucrose concentration, accuracy and precision may be compromised due to the software algorithm that subtracts glucose from sucrose. To avoid compromising accuracy, refer to Application Note 204LS.

III. CALCULATIONS

To calculate % glucose and sucrose, multiply the values reported by the appropriate dilution factor.

Example: 6.65 g of peanut butter was diluted to 100 mL in a Class A volumetric flask. When assayed, the values reported were 1.21 g/L glucose and 3.22 g/L sucrose.

% Glucose: 1.21 g/L x 0.100 L/6.65 g	= 0.0182 g glucose/g peanut butter = 1.82% (w/w)

% Sucrose: 3.22 g/L x 0.100 L/6.65 g	= 0.0484 g sucrose/g peanut butter = 4.84% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900	Biochemistry Analyzer
2365	Glucose Membrane Kit
2776	Glucose Standard Solution (2.50g/L)
2777	Glucose Standard Solution (25.0g/L)
2703	Sucrose Membrane Kit
2780	Sucrose Standard Solution (5.00g/L)
2778	Sucrose Standard Solution (25.0g/L)
2357	Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation) ■

Lactose Measurement in Cheese

INTRODUCTION

Lactose concentrations in complex matrices such as cheese can be measured directly and quickly using the YSI Model 2900 Series Biochemistry Analyzer. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a sample is injected into the sample chamber, the lactose diffuses into the membrane containing galactose oxidase. The lactose is immediately oxidized to hydrogen peroxide and a galactose dialdehyde derivative. 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 lactose concentration.

I. MATERIALS & SETUP

A. YSI Model 2900 Series Biochemistry Analyzer equipped with a 2702 Galactose Oxidase Membrane and 2705 Buffer.

B. Lactose standards (5.00 g/L, 25.0 g/L).

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

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

E. Volumetric glassware (Class A recommended).

F. The following instrument setup is recommended: Sample size: 25 µL

Probe A Parameters		Autocal Parameters	
Chemistry	Lactose	Temperature	1°C
Unit	g/L	Time	30 Min
Calibrator	5.00 g/L	Sample	5 Sam
End Point	30 Sec	Cal Shift	2%

II. LIMITATIONS

Because galactose and other galactosides (raffinose, stachyose) are also substrates of galactose oxidase, the presence of these in the sample will interfere with this analysis.

III. METHOD

A. Determine an appropriate dilution factor. The sample injected should contain 1 to 10 g/L lactose.

B. Accurately weigh the cheese sample to be analyzed.

Transfer the sample to a beaker and add approximately 50 mL deionized water.

Stir the sample over low heat (~55°C) for 10 to 15 minutes.

Transfer the sample to a 100 mL volumetric flask, using deionized water to aid in the complete transfer. Fill the flask to the mark with deionized water and invert to mix.

C. Calibrate the 2900 Series instrument with a 5.00 g/L lactose standard solution.

D. Check the linearity of the membrane at least once a day by injection of a lactose linearity check solution (25.0 g/L). Refer to the Operations Manual for specifications.

E. Assay the sample prepared in B by aspiration into the 2900 series instrument. The linear range of the system is 0.05 to 25.0 g/L lactose. If the value reported exceeds this, further dilution is required.

F. Calibration should be done frequently as described in the Operations Manual.

IV. CALCULATIONS

To calculate % lactose, multiply the reported result by the appropriate dilution factor.

Example: 9.96 g of cheese was prepared as described. When assayed, the value reported was 2.16 g/L lactose.

% Lactose: 2.16 g/L x 0.100 L/9.96 g	= 0.0217 g lactose/cheese = 2.17% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2935 Opaque Buffer Bottle
2702 Galactose Oxidase Membrane Kit
2783 Lactose Standard Solution (5.00 g/L)
2784 Lactose Standard Solution (25.0 g/L)
2705 Buffer Kit
2392 NaCl Solution (for membrane installation) ■

L–Glutamate Determination

INTRODUCTION

L-Glutamate concentrations in complex matrices can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for specific L-glutamate measurement. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a sample is injected into the sample chamber, the glutamate diffuses into the membrane containing glutamate oxidase. The glutamate is immediately oxidized to hydrogen peroxide, α-ketoglutarate, and ammonia. 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 glutamate concentration.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2754 Glutamate Membrane and 2357 Buffer.

B. L-Glutamate standards (5.00 mmol/L, 10.0 mmol/L).

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

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

E. Volumetric glassware (Class A recommended).

F. The following instrument setup is recommended. Sample size:25 µL

Probe A Parameters		Autocal Parameters	
Chemistry	Glutamate	Temperature	1°C
Unit	mmo/L	Time	30 Min
Calibrator	5.00 mmo/L	Sample	2 Sam
End Point	30 Sec	Cal Shift	2%

II. METHOD

A. Total glutamate concentration should not exceed 10.0 mmol/L, as determined in Part D below; otherwise the sample will require further dilution. Use volumetric glassware for all dilutions.

B. Calibrate the 2900 Series instrument with a 5.00 mmol/L L-Glutamate Calibration Standard.

C. Check the linearity of the membrane at least once a day by injection of a glutamate linearity check solution (10.0 mmol/L). Refer to the Operators Manual for specifications.

D. Assay the sample by aspiration into the 2900 Series. The linear range of the system is 0.1 to 10.0 mmol/L glutamate. If the value reported exceeds this, further dilution is required.

E. Calibrate frequently as described in the Operations Manual.

III. CALCULATIONS

To calculate % glutamate, multiply the reported value by the appropriate dilution factor.

For the examples, glutamate concentrations are expressed as monosodium glutamate. The molecular weight of monosodium glutamate (MSG) is 187.13 g/mole or 0.18713 g/mmol.

Example: The contents of a can of soup were blended in a blender on medium speed for about 3 minutes. 10.09 g of blended soup was diluted to 100 mL in a Class A volumetric flask with water. When assayed, the value reported was 1.42 mmol/L glutamate.

% Glutamate: 1.42 mmo/L x 0.100L/10.09g x 0.18713g/mmol MSG	= 0.0027 g MSG/g soup = 0.27% (w/w)

Example: 2.50 grams of a dry powder seasoning mix was diluted to 100 mL in a Class A volumetric flask with water. The mixture was stirred for 5 minutes. When assayed, the value reported was 6.85 mmol/L glutamate.

6.85 mmo/L x 0.100L/2.50g x 0.18713g/mmol MSG	= 0.0512 g MSG/g seasoning mix = 5.12% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2754 Glutamate Oxidase Membrane Kit
2755 L-Glutamate Standard Solution (5.00 mmol/ L)
2756 L-Glutamate Standard Solution (10.0 mmol/ L)
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

Determination of Hydrogen Peroxide

INTRODUCTION

Hydrogen peroxide can be measured quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique technology provides for rapid hydrogen peroxide determination. Measurements are virtually unaffected by color, turbidity, density, or pH.

When a sample is injected into the sample chamber, the hydrogen peroxide diffuses to the platinum electrode and is oxidized. The current flow at the electrode is directly proportional to hydrogen peroxide concentration. The blank membrane placed over the electrode surface rejects potential interfering substances.

Low molecular weight phenols, mercaptans, hydroxylamines, hydrazines, and anilines can be electrochemical interferences. Refer to the Operations Manual for specifics.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2701 Blank Membrane and 2357 Buffer.

B. Hydrogen Peroxide Standards. YSI does not offer hydrogen peroxide standards. Prepare a calibration standard with a hydrogen peroxide concentration near the expected assay value of the sample, however, the calibration standard must produce more than 5 nA of current. In general, about 15 ppm (mg/L) hydrogen peroxide is the lower limit for a calibration standard.

   A linearity standard can also be prepared. Target a concentration that reflects the highest hydrogen peroxide concentration of the samples being analyzed.

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

D. Perform the instrument and membrane check described in the Operations Manual.

E. Volumetric glassware (Class A recommended).

F. The following instrument setup is recommended. Sample size 25 µL*

Probe A Parameters		Autocal Parameters	
Chemistry	Peroxide	Temperature	1°C
Unit	mg/L (ppm)	Time	30 Min
Calibrator	~30 (see section B left)	Sample	5 Sam
End Point	30 Sec	Cal Shift	2%

* The sample volume can be changed to meet the specific needs. Low hydrogen peroxide concentrations will require larger sample volumes.

The calibration solution should be stored and sampled from glass containers. However, if the solution is prepared and used the same day, calibrate from Station #2 and sample from test tubes.

II. METHOD

A. Dilute samples with distilled water to bring the hydrogen peroxide concentration below 300 ppm.

B. Calibrate the 2900 Series instrument with the hydrogen peroxide calibration standard prepared in I.B.

C. If desired, check the linearity of the membrane by injection of the linearity solution prepared in II.B. Typically, hydrogen peroxide response is linear from 3 to 300 ppm.

D. Assay the sample prepared in II.A. by aspiration into the 2900 Series. If the value reported exceeds 300 ppm, dilute the sample further.

E. Calibrate frequently as described in the Operations Manual.

III. CALCULATIONS

To calculate % hydrogen peroxide, multiply the reported value by the appropriate dilution factor.

Example: 10.0 ml of sample was diluted to 100 mL in a Class A volumetric flask. When assayed, the value reported was 220 mg/L (ppm).

Hydrogen Peroxide in original sample:
220 mg/L x 0.100L/0.010L = 2200 mg/L (ppm)

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2701 Blank Membrane Kit
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

Simultaneous Measurement of Glucose and Sucrose in Frozen Ice Cream Bars

INTRODUCTION

Dextrose (D-glucose) and sucrose concentrations in complex matrices such as ice cream bars can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid glucose and sucrose measurements. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a 2900 Series Biochemistry Analyzer is equipped with a glucose and a sucrose membrane, simultaneous measurement of both analytes is possible. Because glucose interferes with sucrose analysis, it is necessary to follow this protocol when analyzing for sucrose in the presence of glucose.

When a sample is injected into the sample chamber, the sucrose diffuses to the sucrose membrane, which contains invertase, mutarotase, and glucose oxidase. The sucrose is hydrolyzed to α-D-glucose and fructose. The mutarotase allows for the quick equilibrium of glucose between its α and β forms. In the presence of glucose oxidase, the β-D-glucose (glucose) is oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The glucose in the sample diffuses to both the glucose and sucrose membranes, which contain glucose oxidase, and is oxidized as well. Subtracting the response of the glucose membrane from the response of the sucrose membrane yields the response due to sucrose alone. The glucose response is taken directly from the glucose membrane. The algorithm in the instrument software calculates the net concentrations. For more information on this system, refer to the Operations Manual.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2703 Sucrose Membrane, a 2365 Glucose Membrane and 2357 Buffer.

B. Glucose (2.50 g/L, 25.00 g/L) and Sucrose (5.00 g/L, 25.0 g/L) standard solutions.

C. Buffer Diluent (40 g/L NaH_2PO_4, 10g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

G. The following instrument setup is recommended: Sample Size 10 µL

Probe A Parameters

Chemistry	Glucose
Unit	g/L
Calibrator	2.50
End Point	30 Sec

Probe B Parameters

Chemistry	Sucrose
Unit	g/L
Calibrator	5.00 g/L
End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

A. Separate the chocolate coating from the ice cream.

B. Transfer the chocolate coating to a container that is water resistant and can be closed, such as a bottle or a plastic bag.

C. Immerse the container into a beaker of hot water (55-65°C) making sure no water enters the container. Allow the sample to melt stirring occasionally to keep the sample homogeneous.

D. Transfer about 5 grams (record exact weight) of the chocolate coating to a 100 mL volumetric flask, using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about 20 minutes.

E. Transfer the ice cream (without coating) into a water resistant container and immerse in hot water until the sample is melted. Stir occasionally to keep the sample homogeneous.

F. Transfer about 10 grams (record exact weight) of the ice cream sample to a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about 20 minutes.

G. Calibrate the 2900 Series instrument with 2.50 g/L glucose and 5.00 g/L sucrose standard solutions.

H. Check the linearity of the membrane at least once a day by injection of glucose (25.00 g/L) and sucrose (25.0 g/L) linearity check solutions. Refer to the Operations Manual for specifications.

I. Shake or stir the sample prepared in D, to keep the sample homogeneous. Assay the sample prepared in D, by aspiration into the 2900 Series instrument. Then assay the sample prepared in F by aspiration into the 2900 Series.*

J. Calibrate frequently as described in the Operations Manual.

* The linear range of the system is 0 to 25.0 g/L for both glucose and sucrose. The combined concentration of glucose + sucrose cannot exceed 25 g/L. If the sum of the values reported exceeds this, further dilution of the sample is required. If the glucose concentration exceeds the sucrose concentration accuracy and precision may be compromised due to the software algorithm that subtracts glucose from sucrose. To avoid compromising accuracy refer to Application Note 204LS.

III. CALCULATIONS

To calculate % glucose and sucrose, multiply the values reported by the appropriate dilution factor.

Example: 10.10 grams of ice cream were diluted to 100 mL in a Class A volumetric flask. When assayed, the values reported were 0.545 g/L glucose and 9.93 g/L sucrose.

% Glucose: 0.545 g/L x 0.100L /10.10 g	= 0.0054 gram glucose/gram ice cream = 0.54% (w/w)

% Sucrose: 9.93 g/L x 0.100L /10.10 g	= 0.0983 gram glucose/gram ice cream = 9.83% (w/w)

Example: 5.01 grams of chocolate coating were diluted to 100 mL in a class A volumetric flask. When assayed, the values reported were 0.118 g/L glucose and 8.73 g/L sucrose.

% Glucose: 0.118 g/L x 0.100L /5.01 g	= 0.0024 gram glucose/gram coating = 0.24% (w/w)

% Sucrose: 8.73 g/L x 0.100L /5.01 g	= 0.1743 gram glucose/gram coating = 17.4% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2365 Glucose Membrane Kit
2776 Glucose Standard Solution (2.50 g/L)
2777 Glucose Standard Solution (25.00 g/L)
2703 Sucrose Membrane Kit
2780 Sucrose Standard Solution (5.00 g/L)
2778 Sucrose Standard Solution (25.0 g/L)
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

Glucose Measurement in Canned Green Beans

INTRODUCTION

Dextrose (D-glucose) concentrations in complex matrices such as canned green beans can be measured directly and quickly 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.

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 hence to glucose concentration.

I. MATERIALS & SETUP

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

B. Glucose standards (2.50 g/L, 9.00 g/L).

C. Buffer Diluent (40 g/L NaH_2PO_4, 10 g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

G. 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 g/L	Sample	5 Sam
End Point	30 Sec	Cal Shift	2%

II. METHOD

A. Empty contents of can into a clean dry blender.

B. Blend until the sample is liquid, about ten minutes.

C. Transfer 25 to 50 grams of the blended sample to a 100 mL volumetric flask, using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about twenty minutes before analysis.

D. Calibrate the 2900 Series instrument with a 2.50 g/L glucose standard solution.

E. 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.

F. Assay the sample prepared in C by aspiration into the 2900 Series. The linear range of the system is 0.05 to 9.00 g/L glucose. If the value reported exceeds this, further dilution is required.*

G. Calibrate frequently as described in the Operations Manual.

* The linearity of glucose on the 2900 series instrument can be increased to 0.05 to 25.0 g/L. This can be done by decreasing the sample size to 10 µL and checking the linearity with a 25.0 g/L solution.

III. CALCULATIONS

To calculate % glucose, multiply the reported value by the appropriate dilution factor.

Example: 50.02 grams of blended green beans were diluted to 100 mL in a Class A volumetric flask. When assayed, the value reported was 2.93 g/L glucose.

% Glucose: 2.93 g/L x 0.100L /50.02 g	= 0.0059 gram glucose/gram green beans = 0.59% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2365 Glucose Membrane Kit
2776 Glucose Standard Solution (2.50 g/L)
1531 Glucose Standard Solution (9.00 g/L)
2777 Glucose Standard Solution (25.0 g/L)
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

Glucose Measurement in Frozen Green Beans

INTRODUCTION

Dextrose (D-glucose) concentrations in complex matrices such as frozen green beans can be measured directly and quickly 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.

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 hence to glucose concentration.

I. MATERIALS & SETUP

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

B. Glucose standards (2.50 g/L, 9.00 g/L).

C. Buffer Diluent (40 g/L NaH_2PO_4, 10 g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

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

Probe A Parameters

Chemistry	Glucose
Unit	g/L
Calibrator	2.50 g/L
End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

A. Weigh up to 25.00 g of green beans to be analyzed, add 50 mL of buffer.

B. Transfer the sample a clean dry blender. Blend until the sample is liquid.

C. Transfer the sample to a 100mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer. Allow the solution to equilibrate for about twenty minutes before analysis.

D. Calibrate the 2900 Series instrument with a 2.50 g/L glucose standard solution.

E. 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.

F. Assay the sample prepared in C by aspiration into the 2900 Series. The linear range of the system is 0.05 to 9.00 g/L glucose. If the value reported exceeds this, further dilution is required.*

G. Calibrate frequently as described in the Operations Manual.

* The linearity of glucose on the 2900 Series can be increased to 0.05 to 25.0 g/L. This can be done by decreasing the sample size to 10µL and checking the linearity with 25.0 g/L standard.

III. CALCULATIONS

To calculate % glucose, multiply the reported value by the appropriate dilution factor.

Example: 27.22 grams of frozen green beans were diluted to 100 mL in a Class A volumetric flask. When assayed, the value reported was 2.20 g/L glucose.

% Glucose: 2.20 g/L x 0.100L /27.22 g	= 0.0081 g glucose/ g green beans = 0.81% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2365 Glucose Membrane Kit
2776 Glucose Standard Solution (2.50 g/L)
1531 Glucose Standard Solution (9.00 g/L)
2777 Glucose Standard Solution (25.0 g/L)
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

L-Lactate in Lunch Meats

INTRODUCTION

L-Lactate concentrations in complex matrices such as lunch meats can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for specific L-lactate measurement. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a sample is injected into the sample chamber, the L-lactate diffuses into the membrane containing L-lactate oxidase. The L-lactate is immediately oxidized to hydrogen peroxide and pyruvate. 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 L-lactate concentration.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2329 L-Lactate Membrane and 2357 Buffer.

B. L-Lactate standards (0.50 g/L, 2.50 g/L).

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

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

E. Volumetric glassware (Class A recommended).

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

Probe A Parameters

Chemistry	L-Lactate
Unit	g/L
Calibrator	0.50 g/L
End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

A. Cut the sample into several pieces (about 1 inch squares).

B. Weigh the cut pieces of meat and record the exact weight.

C. Transfer the sample to a clean dry blender. Add about 100 mL of distilled or deionized water. Turn the blender on and allow the sample to blend for about 5 minutes.

D. Transfer the sample to a 500 mL volumetric flask. Using distilled or deionized water, rinse the blender and use this rinse to dilute the sample to the mark on the flask.

E. Calibrate the 2900 Series instrument with a 0.50 g/L L-lactate standard solution.

F. Assay the sample prepared in D by aspiration into the 2900 Series. The linear range of the system extends to 2.50 g/L L-lactate. If the value reported exceeds this, further dilution is required.

G. Check the linearity of the membrane at least once a day by injection of an L-lactate linearity check solution (2.50 g/L). Refer to the Operations Manual for specifications.

H. Calibrate frequently as described in the Operations Manual.

III. CALCULATIONS

27.93 grams of turkey lunch meat was diluted to 500 mL in a Class A volumetric flask. When assayed the value reported was 1.27 g/L L-lactate.

% L-Lactate 1.27 g/L x 0.500L /27.93 g	= 0.0227 g L-Lactate/g Meat = 2.27% (w/w)

To measure sodium lactate multiply the concentration by the ratio of the formula weights of sodium lactate and L-lactate.

Sodium lactate L-lactate 112.07 / 89.07 2.27%(w/w) L-lactate x 1.26	= 112.07 g/mole = 89.07 g/mole = 1.26 = 2.86% (w/w) sodium lactate

ORDERING INFORMATION

YSI Part Numbers:

2900	Biochemistry Analyzer
2329	L-Lactate Membrane Kit
2776	L-Lactate Standard Solution (0.50 g/L)
2777	L-Lactate Standard Solution (2.50 g/L)
2357	Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation) ■

Simultaneous Measurement of Glucose and Sucrose in Corn and Peas

INTRODUCTION

Glucose (D-glucose) and sucrose concentrations in complex matrices such as corn and peas can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid glucose and sucrose measurements. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a 2900 Series Biochemistry Analyzer is equipped with a glucose and a sucrose membrane, simultaneous measurement of both analytes is possible. Because glucose interferes with sucrose analysis, it is necessary to follow this protocol when analyzing for sucrose in the presence of glucose.

When a sample is injected into the sample chamber, the sucrose diffuses to the sucrose membrane, which contains invertase, mutarotase, and glucose oxidase. The sucrose is hydrolyzed to α-D-glucose and fructose. The mutarotase allows for the quick equilibrium of glucose between its α and β forms. In the presence of glucose oxidase, the β-D-glucose (glucose) is oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The glucose in the sample diffuses to both the glucose and sucrose membranes, which contain glucose oxidase, and is oxidized as well. Subtracting the response of the glucose membrane from the response of the sucrose membrane yields the response due to sucrose alone. The glucose response is taken directly from the glucose membrane. The algorithm in the instrument software calculates the net concentrations. For more information on this system, refer to the Operations Manual.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2703 Sucrose Membrane, a 2365 Glucose Membrane and 2357 Buffer.

B. Glucose (2.50 g/L, 25.00 g/L) and Sucrose (5.00 g/L, 25.0 g/L) standard solutions.

C. Buffer Diluent (40 g/L NaH_2PO_4, 10g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

G. The following instrument setup is recommended: Sample Size 10 µL

Probe A Parameters	
Chemistry	Glucose
Unit	g/L
Calibrator	2.50
End Point	30 Sec

Probe B Parameters	
Chemistry	Sucrose
Unit	g/L
Calibrator	5.00 g/L
End Point	30 Sec

Autocal Parameters	
Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

A. Weigh up to 25.00 grams of corn or peas to be analyzed. Add about 50 mL buffer diluent.

B. Transfer the solution to a clean dry blender. Blend until the sample is liquid.

C. Transfer the sample to a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about twenty minutes before analysis.

D. Calibrate the 2900 Series instrument with a 2.50 g/L glucose and 5.00 g/L sucrose standard solutions.

E. Check the linearity of the membranes at least once a day by injection of glucose (25.00 g/L) and sucrose (25.0 g/L) linearity check solutions. Refer to the Operations Manual for specifications.

F. Assay the sample prepared in C by aspiration into the 2900 Series instrument.*

G. Calibrate frequently as described in the Operations Manual.

* The linear range of the system is 0 to 25.0 g/L for both glucose and sucrose. The combined concentration of glucose + sucrose cannot exceed 25 g/L. If the sum of the values reported exceeds this, further dilution of the sample is required.

If the glucose concentration exceeds the sucrose concentration, accuracy and precision may be compromised due to the software algorithm that subtracts glucose from sucrose. To avoid compromising accuracy refer to Application Note 204LS.

III. CALCULATIONS

To calculate % glucose and sucrose, multiply the values reported by the appropriate dilution factor.

Example: 25.10 grams of peas were diluted to 100 mL in a Class A volumetric flask. When assayed, the values reported were 0.128 g/L glucose and 6.91 g/L sucrose.

% Glucose: 0.128 g/L x 0.100L /25.10 g	= 0.0005 g glucose/g peas = 0.05% (w/w)

% Sucrose: 6.91 g/L x 0.100L /25.10 g	= 0.0275 g sucrose/g peas = 2.75% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900	Biochemistry Analyzer
2365	Glucose Membrane Kit
2776	Glucose Standard Solution (2.50 g/L)
2777	Glucose Standard Solution (25.00 g/L)
2703	Sucrose Membrane Kit
2780	Sucrose Standard Solution (5.00 g/L)
2778	Sucrose Standard Solution (25.0 g/L)
2357	Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation) ■

Simultaneous Measurement of Glucose and Sucrose in Cereal Products

INTRODUCTION

Dextrose (D-glucose) and sucrose concentrations in complex matrices such as cereal products can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for specific glucose and sucrose measurements. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a 2900 Series Biochemistry Analyzer is equipped with a glucose and a sucrose membrane, simultaneous measurement of both analytes is possible. Because glucose interferes with sucrose analysis, it is necessary to follow this protocol when analyzing for sucrose in the presence of glucose.

When a sample is injected into the sample chamber, the sucrose diffuses to the sucrose membrane, which contains invertase, mutarotase, and glucose oxidase. The sucrose is hydrolyzed to α-D-glucose and fructose. The mutarotase allows for the quick equilibrium of glucose between its α and β forms. In the presence of glucose oxidase, the β-D-glucose (glucose) is oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The glucose in the sample diffuses to both the glucose and sucrose membranes, which contain glucose oxidase, and is oxidized as well. Subtracting the response of the glucose membrane from the response of the sucrose membrane yields the response due to sucrose alone. The glucose response is taken directly from the glucose membrane. The algorithm in the instrument software calculates the net concentrations. For more information on this system, refer to the Operations Manual.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2703 Sucrose Membrane, a 2365 Glucose Membrane and 2357 Buffer.

B. Glucose (2.50 g/L,25.00 g/L) and Sucrose (5.00 g/L, 25.0 g/L) standard solutions.

C. Buffer Diluent (40 g/L NaH_2PO_4, 10g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

G. The following instrument setup is recommended: Sample Size 10 µL

Probe A Parameters		Probe B Parameters	
Chemistry	Glucose	Chemistry	Sucrose
Unit	g/L	Unit	g/L
Calibrator	2.50	Calibrator	5.00 g/L
End Point	30 Sec	End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

A. Grind sample to a fine powder.

B. Weigh 1.000 to 5.000 g of powdered sample.

C. Transfer the sample to a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about twenty minutes before analysis.

D. Calibrate the 2900 Series instrument with a 2.50 g/L glucose and 5.00 g/L sucrose standard solutions.

E. Check the linearity of the membranes at least once a day by injection of glucose (25.00 g/L) and sucrose (25.0 g/L) linearity check solutions. Refer to the Operations Manual for specifications.

F. Assay the sample prepared in B by aspiration into the 2900 Series instrument.*

G. Calibrate frequently as described in the Operations Manual.

* The linear range of the system is 0 to 25.0 g/L for both glucose and sucrose. The combined concentration of glucose + sucrose cannot exceed 25 g/L. If the sum of the values reported exceeds this, further dilution of the sample is required. If the glucose concentration exceeds the sucrose concentration, accuracy and precision may be compromised due to the software algorithm that subtracts glucose from sucrose. To avoid compromising accuracy refer to Application Note 204LS.

III. CALCULATIONS

To calculate % glucose and sucrose, multiply the values reported by the appropriate dilution factor.

Example: A cereal sample (4.336 g) was prepared and assayed as described. The values reported were 0.62 g/L glucose and 9.88 g/L sucrose.

% Glucose: 0.62 g/L x 0.100L /4.336 g	= 0.0143 g glucose/g cereal = 1.43% (w/w)

% Sucrose: 9.88 g/L x 0.100L /4.336 g	= 0.2279 g sucrose/g cereal = 22.8% (w/w)

IV. SAMPLES TESTED

Several cereal samples were assayed using YSI technology and HPLC. The results are listed below.

	Sucrose (%)		Glucose (%)		
Sample	YSI	HPLC	YSI	HPLC	Label (%)*
A	35.0	36.0	1.24	0.96	38.8
B	21.4	22.2	0.85	0.50	21.1
C	22.4	23.1	1.52	1.52	24.6
D	17.4	18.3	0.27	0.07	28.0
E	25.2	25.4	1.61	1.33	28.2
F	29.8	27.9	3.51	4.14	35.2
G	32.2	31.1	3.01	2.42	38.7
H	32.9	30.4	0.71	0.43	38.7
I	5.40	6.70	1.03	0.70	7.0

* Label information reported as "Sucrose and Other Sugars."

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2365 Glucose Membrane Kit
2776 Glucose Standard Solution (2.50 g/L)
2777 Glucose Standard Solution (25.00 g/L)
2703 Sucrose Membrane Kit
2780 Sucrose Standard Solution (5.00 g/L)
2778 Sucrose Standard Solution (25.0 g/L)
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

Simultaneous Measurement of Glucose and Sucrose in Baked Goods

INTRODUCTION

Dextrose (D-glucose) and sucrose concentrations in complex matrices such as baked goods can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid glucose and sucrose measurements. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a 2900 Series Biochemistry Analyzer is equipped with a glucose and a sucrose membrane, simultaneous measurement of both analytes is possible. Because glucose interferes with sucrose analysis, it is necessary to follow this protocol when analyzing for sucrose in the presence of glucose.

When a sample is injected into the sample chamber, the sucrose diffuses to the sucrose membrane, which contains invertase, mutarotase, and glucose oxidase. The sucrose is hydrolyzed to α-D-glucose and fructose. The mutarotase allows for the quick equilibrium of glucose between its α and β forms. In the presence of glucose oxidase, the β-D-glucose (glucose) is oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The glucose in the sample diffuses to both the glucose and sucrose membranes, which contain glucose oxidase, and is oxidized as well. Subtracting the response of the glucose membrane from the response of the sucrose membrane yields the response due to sucrose alone. The glucose response is taken directly from the glucose membrane. The algorithm in the instrument software calculates the net concentrations. For more information on this system, refer to the operations manual.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2703 Sucrose Membrane, a 2365 Glucose Membrane and 2357 Buffer.

B. Glucose (2.50 g/L, 25.00 g/L) and Sucrose (5.00 g/L, 25.0 g/L) standard solutions.

C. Buffer Diluent (40 g/L NaH_2PO_4, 10g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

G. The following instrument setup is recommended: Sample Size 10 µL

Probe A Parameters

Chemistry	Glucose
Unit	g/L
Calibrator	2.50
End Point	30 Sec

Probe B Parameters

Chemistry	Sucrose
Unit	g/L
Calibrator	5.00 g/L
End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

A. Grind sample to a fine powder.

B. Weigh 1.00 to 5.00 g of powdered sample.

C. Transfer the sample to a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about twenty minutes before analysis.

D. Calibrate the 2900 Series instrument with 2.50 g/L glucose and 5.00 g/L sucrose standard solutions.

E. Check the linearity of the membranes at least once a day by injection of glucose (25.0 g/L) and sucrose (25.0 g/L) linearity check solutions. Refer to the Operations Manual for specifications.

F. Assay the sample prepared in B by aspiration into the 2900 Series instrument.*

G. Calibrate frequently as described in the Operations Manual.

* The linear range of the system is 0 to 25.0 g/L for both glucose and sucrose. The combined concentration of glucose + sucrose cannot exceed 25 g/L. If the sum of the values reported exceeds this, further dilution of the sample is required. If the glucose concentration exceeds the sucrose concentration, accuracy and precision may be compromised due to the software algorithm that subtracts glucose from sucrose. To avoid compromising accuracy refer to Application Note 204LS.

III. CALCULATIONS

To calculate % glucose and sucrose, multiply the values reported by the appropriate dilution factor.

Example: A baked muffin sample (4.654 g) was prepared and assayed as described. The values reported were 0.81 g/L glucose and 8.54 g/L sucrose.

% Glucose: 0.81 g/L x 0.100L /4.654 g	= 0.0174 g glucose/g muffin = 1.74% (w/w)

% Sucrose: 8.54 g/L x 0.100L /4.654 g	= 0.1834 g sucrose/g muffin = 18.3% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900	Biochemistry Analyzer
2365	Glucose Membrane Kit
2776	Glucose Standard Solution (2.50 g/L)
2777	Glucose Standard Solution (25.0 g/L)
2703	Sucrose Membrane Kit
2780	Sucrose Standard Solution (5.00 g/L)
2778	Sucrose Standard Solution (25.0 g/L)
2357	Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation) ■

Simultaneous Measurement of Glucose and Sucrose in Sweetened Condensed Milk

INTRODUCTION

Dextrose (D-glucose) and sucrose concentrations in complex matrices such as sweetened condensed milk can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid glucose and sucrose measurements. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a 2900 Series Biochemistry Analyzer is equipped with a glucose and a sucrose membrane, simultaneous measurement of both analytes is possible. Because glucose interferes with sucrose analysis, it is necessary to follow this protocol when analyzing for sucrose in the presence of glucose.

When a sample is injected into the sample chamber, the sucrose diffuses to the sucrose membrane, which contains invertase, mutarotase, and glucose oxidase. The sucrose is hydrolyzed to α-D-glucose and fructose. The mutarotase allows for the quick equilibrium of glucose between its α and β forms. In the presence of glucose oxidase, the β-D-dextrose (glucose) is oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The glucose in the sample diffuses to both the glucose and sucrose membranes, which contain glucose oxidase, and is oxidized as well. Subtracting the response of the glucose membrane from the response of the sucrose membrane yields the response due to sucrose alone. The glucose response is taken directly from the glucose membrane. The algorithm in the instrument software calculates the net concentrations. For more information on this system, refer to the Operations Manual.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2703 Sucrose Membrane, a 2365 Glucose Membrane and 2357 Buffer.

B. Glucose (2.50 g/L, 25.00 g/L) and Sucrose (5.00 g/L, 25.0 g/L) standard solutions.

C. Buffer Diluent (40 g/L NaH_2PO_4, 10g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

G. The following instrument setup is recommended: Sample Size 10 µL

Probe A Parameters

Chemistry	Glucose
Unit	g/L
Calibrator	2.50
End Point	30 Sec

Probe B Parameters

Chemistry	Sucrose
Unit	g/L
Calibrator	5.00 g/L
End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

A. As with all applications, make sure the sample is homogeneous. In the case of a canned sample of sweetened condensed milk, warm the can in a water bath that is between 35-45°C. Shake the can before opening. Transfer the contents of the can to another container. Scrape the top, bottom and sides of the can, making sure to remove any sugar that may have settled. Stir entire contents.

B. Weigh up to 1.000 g of sweetened condensed milk to be analyzed.

C. Transfer the sample to a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about twenty minutes before analysis.

D. Calibrate the 2900 Series instrument with 2.50 g/L glucose and 5.00 g/L sucrose standard solutions.

E. Check the linearity of the membranes at least once a day by injection of glucose (25.0 g/L) and sucrose (25.0 g/L) linearity check solutions. Refer to the Operations Manual for specifications.

F. Assay the sample prepared in B by aspiration into the 2900 Series instrument.*

G. Calibrate frequently as described in the Operations Manual.

* The linear range of the system is 0.05 to 25.0 g/L for both glucose and sucrose. The combined concentration of glucose + sucrose cannot exceed 25.0 g/L. If the sum of the values reported exceeds this, further dilution of the sample is required.

If the glucose concentration exceeds the sucrose concentration, accuracy and precision may be compromised due to the software algorithm that subtracts glucose from sucrose. To avoid compromising accuracy, refer to Application Note 204LS.

III. CALCULATIONS

To calculate % glucose and sucrose, multiply the values reported by the appropriate dilution factor.

Example: 1.0135 g of sweetened condensed milk was diluted to 100 mL in a Class A volumetric flask. When assayed, the values reported were 0.096 g/L glucose and 4.47 g/L sucrose.

% Glucose: 0.096 g/L x 0.100L /1.0135 g	= 0.0095 g glucose/g milk = 0.95% (w/w)

% Sucrose: 4.47 g/L x 0.100L /1.0135 g	= 0.4410 g sucrose/g milk = 44.1% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2365 Glucose Membrane Kit
2776 Glucose Standard Solution (2.50 g/L)
2777 Glucose Standard Solution (25.0 g/L)
2703 Sucrose Membrane Kit
2780 Sucrose Standard Solution (5.00 g/L)
2778 Sucrose Standard Solution (25.0 g/L)
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

Glucose Measurement in Corn Syrup and Other Syrup Products

INTRODUCTION

Dextrose (D-glucose) concentrations in complex matrices such as corn syrup can be measured directly and quickly 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.

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 hence to dextrose concentration.

I. MATERIALS & SETUP

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

B. Glucose standards (2.50 g/L, 9.00 g/L).

C. Buffer Diluent (40 g/L NaH_2PO_4, 10g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

G. 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. METHOD

A. Weigh 0.500 to 5.000 g of the corn syrup to be analyzed.

B. Transfer the sample to a 100 mL volumetric flask, using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about twenty minutes before analysis.

C. Calibrate the 2900 Series instrument with a 2.50 g/L glucose standard solution.

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

E. Assay the sample prepared in B by aspiration into the 2900 Series instrument. The linear range of the system is 0.05 to 9.00 g/L glucose. If the value reported exceeds this, further dilution is required.*

F. Calibrate frequently as described in the Operations Manual.

* The glucose linearity on the 2900 Series may be increased to 0.05 to 25.0 g/L. This can be done by decreasing the sample size to 10µL and checking the linearity with a 25.0 g/L standard.

III. CALCULATIONS

To calculate % glucose, multiply the reported value by the appropriate dilution factor.

Example: 2.555 g of corn syrup was diluted to 100 mL in a Class A volumetric flask. When assayed, the value reported was 4.65 g/L glucose.

% Glucose: 4.65 g/L x 0.100L /2.555 g	= 0.1820 g glucose/g corn syrup = 18.2% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2365 Glucose Membrane Kit
2776 Glucose Standard Solution (2.50 g/L)
1531 Glucose Standard Solution (9.00 g/L)
2777 Glucose Standard Solution (25.0 g/L)
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

Measurement of Glucose and Sucrose in Potatoes

INTRODUCTION

Dextrose (D-glucose) and sucrose concentrations in complex matrices such as potatoes can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid dextrose and sucrose measurement. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a YSI 2900 Series Biochemistry Analyzer is equipped with a dextrose membrane, both sucrose and dextrose concentrations can be measured. This is accomplished by first determining the glucose concentration. The sucrose is then hydrolyzed to dextrose, and the total dextrose concentration is measured. The difference in the responses corresponds to the sucrose concentration.

After a sample is injected into the sample chamber, the dextrose diffuses to the glucose membrane, which contains glucose oxidase, and is oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode. The current produced is directly proportional to the hydrogen peroxide and dextrose concentrations. For more information on this system, refer to the Operations Manual.

I. MATERIALS & SETUP

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

B. Glucose standards (2.50 g/L, 9.00 g/L).

C. Buffer Diluent (40 g/L NaH_2PO_4, 10g/L Na_2HPO_4 in reagent water).

D. Invertase - Sigma Chemical Company I-4504 recommended.

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

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

G. Volumetric glassware (Class A recommended).

H. 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. METHOD

A. Weigh 100 to 200 grams of washed and peeled potatoes. For information on sample selection, see J. R. Sowokinos, *American Potato Journal*, 50, 333-334 (1978).

B. Juicerate the potatoes in an Acme Juicerator and collect the juice in a beaker. Wash the juicerator three times with 100 mL portions of buffer diluent. Wait two to three minutes between washings.

C. Quantitatively transfer the combined juice and buffer to a 500 mL volumetric flask. Rinse the beaker with several small (10 mL) aliquots of buffer and transfer to the flask. Dilute to the mark with buffer. Refrigerate for one hour prior to analysis.*

D. Remove about 3 mL of the solution from C and add ~2 mg of invertase enzyme. Stir gently until dissolved. Cover the sample and allow incubation at room temperature for 20 minutes before analysis.

E. Calibrate the 2900 series instrument witha 2.50 g/L glucose standard solution.

F. 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 Operations Manual for specifications.

G. Assay the sample prepared in C by aspiration into the 2900 Series. This is the free glucose concentration (D_{free}).**

H. Assay the sample prepared in D (with invertase). The value reported is the sum of the free glucose and that produced from sucrose hydrolysis (D_{total}).

I. Calibrate frequently as described in the Operations Manual.

* For potato samples with low glucose and sucrose levels, consider increasing the ratio of potato sample to the volume of extracting buffer, or consider increasing the sample size aspirated into the instrument (II.H).

** The linear range of the 2900 Series instrument may be increased to 25.0 g/L. This can be done by decreasing the sample size to 10 µL and checking the linearity with a 25.0 g/L standard.

III. CALCULATIONS

Example: A 223 g potato sample was prepared as described in II. When the sample from II.C was assayed, the value reported (D_{free}) was 2.13 g/L glucose. The value reported for the sample from II.D (with invertase) was 3.36 g/L dextrose (D_{total}).

To calculate % glucose, multiply the reported value (D_{free}) by the appropriate dilution factor.

To calculate % sucrose, subtract D_{free} from D_{total} and multiply by the appropriate dilution and mass ratio factors.

% Glucose: 2.13 g/L x 0.500 L/223 g	= 0.00478 g glucose/g potatoes = 0.48% (w/w)

% Sucrose:

$$(3.36\ g/L - 2.13\ g/L) \times \frac{0.500\ L}{223\ g} \times \frac{342.30\ g/mole\ sucrose}{180.16\ g/mole\ dextrose}$$

= 0.00524 g sucrose/g potatoes

= 0.52% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900	Biochemistry Analyzer
2365	Glucose Membrane Kit
2776	Glucose Standard Solution (2.50 g/L)
2777	Glucose Standard Solution (25.0 g/L)
1531	Glucose Standard Solution (9.00 g/L)
2357	Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation) ■

Dextrose Measurement in Potatoes

INTRODUCTION

Dextrose (D-glucose) concentrations in complex matrices such as potatoes can be measured directly and quickly using the YSI 2900 Series 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.

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 dextrose concentration.

I. MATERIALS & SETUP

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

B. Dextrose standards (2.50 g/L, 9.00 g/L).

C. Buffer Diluent (40 g/L NaH_2PO_4, 10g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

G. 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. METHOD

A. Weigh 100 to 200 grams of washed and peeled potatoes. For information on sample selection, see J. R. Sowokinos, *American Potato Journal*, 50, 333-334 (1978).

B. Juicerate the potatoes in an Acme Juicerator and collect the juice in a beaker. Wash the juicerator three times with 100 mL portions of buffer diluent. Wait two to three minutes between washings.

C. Quantitatively transfer the combined juice and buffer to a 500 mL volumetric flask. Rinse the beaker with several small (10 mL) aliquots of buffer and transfer to the flask. Dilute to the mark with buffer. Refrigerate for one hour prior to analysis.*

D. Calibrate the 2900 series instrument 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 Operations Manual for specifications.

F. Assay the sample prepared in C by aspiration into the 2900 Series. The linear range of the system is 0.05 to 9.00 g/L dextrose. If the value reported exceeds this, further dilution is required.*

G. Calibrate frequently as described in the Operations Manual.

* For potato samples with low dextrose content, consider increasing the ratio of potato sample to the volume of extracting buffer. For higher dextrose levels, more dilute samples are recommended.

The dextrose linearity of the 2900 series may be increased to 0.05 to 25.0 g/L. This can be done by decreasing the samples size to 10 µL and checking the linearity with a 25.0 g/L standard.

III. CALCULATIONS

To calculate % dextrose, multiply the reported value by the appropriate dilution factor.

Example: A 200 g potato sample was prepared as described in III.B and C. When assayed, the value reported was 2.15 g/L dextrose.

% Dextrose: 2.15 g/L x 0.500 L/200 g	= 0.00538 g dextrose/g potatoes = 0.54% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2365 Glucose Membrane Kit
2776 Glucose Standard Solution (2.50 g/L)
1531 Glucose Standard Solution (9.00 g/L)
2777 Glucose Standard Solution (25.0 g/L)
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

Simultaneous Measurement of Dextrose and Sucrose in Molasses

INTRODUCTION

Dextrose (D-glucose) and sucrose concentrations in complex matrices such as molasses can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid dextrose and sucrose measurements. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a 2900 Series Biochemistry Analyzer is equipped with a dextrose and a sucrose membrane, simultaneous measurement of both analytes is possible. Because dextrose interferes with sucrose analysis, it is necessary to follow this protocol when analyzing for sucrose in the presence of dextrose.

When a sample is injected into the sample chamber, the sucrose diffuses to the sucrose membrane, which contains invertase, mutarotase, and glucose oxidase. The sucrose is hydrolyzed to α-D-glucose and fructose. The mutarotase allows for the quick equilibrium of glucose between the α and β forms. In the presence of glucose oxidase, the β-D-glucose is oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The dextrose in the sample diffuses to both the dextrose and sucrose membranes, which contain glucose oxidase, and is oxidized as well. Subtracting the response of the dextrose membrane from the response of the sucrose membrane yields the response due to sucrose alone. The dextrose response is taken directly from the dextrose membrane. The algorithm in the instrument software calculates the net concentrations. For more information on this system, refer to the Operations Manual.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2703 Sucrose Membrane, a 2365 Dextrose Membrane and 2357 Buffer.

B. Dextrose (2.50 g/L, 9.00 g/L) and Sucrose (5.00 g/L, 25.0 g/L) standard solutions.

C. Buffer Diluent (40 g/L NaH_2PO_4, 10g/L Na_2HPO_4 in reagent water).

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

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

F. Volumetric glassware (Class A recommended).

G. The injection volume should be set to 25µL.

H. The following instrument setup is recommended.

Probe A Parameters

Chemistry	Glucose
Unit	g/L
Calibrator	2.50
End Point	30 Sec

Probe B Parameters

Chemistry	Sucrose
Unit	g/L
Calibrator	5.00 g/L
End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

A. Weigh up to 5.000 g of molasses to be analyzed.

B. Transfer the sample to a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about twenty minutes before analysis.

C. Calibrate the 2900 Series instrument with 2.50 g/L dextrose and 5.00 g/L sucrose standard solutions.

D. Check the linearity of the membrane at least once a day by injection of dextrose (9.00 g/L) and sucrose

linearity check solutions (25.0 g/L). Refer to the Operations Manual for specifications.

E. Assay the sample prepared in B by aspiration into the 2900 Series instrument.*

F. Calibrate frequently as described in the Operations Manual.

* The combined concentration of dextrose + sucrose cannot exceed 25 g/L. If the sum of the values reported exceeds this, further dilution of the sample is required.

If the dextrose concentration exceeds the sucrose concentration, accuracy and precision may be compromised due to the software algorithm that subtracts dextrose from sucrose. To avoid compromising accuracy, refer to Application Note 204LS.

III. CALCULATIONS

To calculate % dextrose and sucrose, multiply the values reported by the appropriate dilution factor.

Example: 4.569 g of molasses was diluted to 100 mL in a Class A volumetric flask. When assayed, the values reported were 6.75 g/L dextrose and 12.94 g/L sucrose.

% Dextrose: 6.75 g/L x 0.100L /4.569 g	= 0.1477 g dextrose/g molasses = 14.8% (w/w)

% Sucrose: 12.94 g/L x 0.100L /4.569 g	= 0.2832 g sucrose/g molasses = 28.3% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900	Biochemistry Analyzer
2365	Dextrose Membrane Kit
2776	Dextrose Standard Solution (2.50 g/L)
1531	Dextrose Standard Solution (9.00 g/L)
2703	Sucrose Membrane Kit
2780	Sucrose Standard Solution (5.00 g/L)
2778	Sucrose Standard Solution (25.0 g/L)
2357	Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation) ■

Sucrose Measurement in Molasses

INTRODUCTION

Sucrose concentrations in complex matrices such as molasses can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid sucrose measurement. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a sample is injected into the sample chamber, the sucrose diffuses into the membrane containing invertase, mutarotase, and glucose oxidase. The sucrose is hydrolyzed to α-D-glucose and fructose. The mutarotase allows for the quick equilibrium of glucose between its α and β forms. In the presence of glucose oxidase, the β-D-glucose (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 through the series of reactions described above, the hydrogen peroxide concentration is also directly proportional to the sucrose concentration.

Because the membrane contains glucose oxidase, any dextrose in the sample will also be oxidized and produce a signal. For this reason, the sample must be dextrose-free. If dextrose is present in the sample, refer to Application Note 220LS for the Simultaneous Measurement of Dextrose and Sucrose in Molasses. For more information, refer to the Operations Manual.

I. MATERIALS & SETUP

A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2703 Sucrose Membrane and 2357 Buffer.

B. Sucrose standards (5.00 g/L25.0 g/L).

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

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

E. Volumetric glassware (Class A recommended).

F. The following instrument setup is recommended. Sample size 25 µL

Probe A Parameters		Autocal Parameters	
Chemistry	Sucrose	Temperature	1°C
Unit	g/L	Time	30 Min
Calibrator	5.00	Sample	5 Sam
End Point	30 Sec	Cal Shift	2%

II. METHOD

A. Weigh up to 5.000 g of molasses to be analyzed.

B. Transfer the sample to a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix.

C. Calibrate the 2900 Series instrument with a 5.00 g/L sucrose standard solution.

D. Check the linearity of the membrane at least once a day by injection of sucrose linearity check solutions (25.00 g/L). Refer to the Operations Manual for specifications.

E. Assay the sample prepared in B by aspiration into the 2900 Series. The linear range of the system is 0.1 to 25.00 g/L sucrose. If the value reported exceeds this, further dilution is required.

F. Calibrate frequently as described in the Operations Manual.

III. CALCULATIONS

To calculate % sucrose, multiply the reported value by the appropriate dilution factor.

Example: 2.001 g of molasses was diluted to 100 mL in a Class A volumetric flask. When assayed, the value reported was 5.89 g/L sucrose.

% Sucrose: 5.89 g/L x 0.100L /2.001 g	= 0.294 g sucrose/g molasses = 29.4% (w/w)

ORDERING INFORMATION

YSI Part Numbers:

2900 Biochemistry Analyzer
2703 Sucrose Membrane Kit
2780 Sucrose Standard Solution (5.00 g/L)
2778 Sucrose Standard Solution (25.0 g/L)
2357 Buffer Kit
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation) ■

Determination of % Cook in Extruded Cereal Products

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. The samples containing starch are treated identically with glucoamylase. Glucose produced from this reaction is measured with the YSI 2900 Series instrument. The ratio of glucose in the cold water sample to glucose in the autoclaved 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 hence to the glucose concentration.

I. MATERIALS & SETUP

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

B. Glucose standards (2.50 g/L, 9.00 g/L).

C. 1N Acetate buffer.

D. Glucoamylase solution.

E. 25% Trichloracetic Acid.

F. Phosphate diluent buffer (40 g/L NaH_2PO_4, 10 g/L Na_2HPO_4 in reagent water, pH 5.9).

G. Volumetric glassware (Class A recommended).

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

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

J. 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. 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% glucoamylase solution
4. Cover with aluminum foil and incubate, in water bath, for one hour at 40°C.
5. Add ~ 3.2 mL of 25% Trichloracetic 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 glucoamylase may contain free glucose, 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 2900 Series instrument with a 2.50 g/L glucose standard solution.

E. 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.

F. Determination of Blank: Assay the blank prepared in C by aspiration into the 2900 Series instrument.*

G. Determination of Cooked Starch: Assay the sample prepared in A by aspiration into the 2900 Series Instrument.*

H. Determination of Total Starch: Assay the sample prepared in B by aspiration into the 2900 Series Instrument.*

I. Calibrate frequently as described in the Operations Manual.

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

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 100 mL volumetric flask and dilute to the mark with phosphate diluent buffer. Mix the sample until dissolved and analyze.

III. 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 glucose is produced when 1.0 g of starch is hydrolyzed, the glucose concentration of the sample should be multiplied by 0.9.

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

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 glucose.

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 glucose.

The blank contained 0.01 g/L of glucose.

Cold water starch: 1.45-0.01 g/L x 0.9 x 0.100L/0.52 g	= 0.249 g starch/g food = 24.9% (w/w)
Total starch: 1.82-0.01 g/L x 0.9 x 0.100L/0.52 g	= 0.313 g starch/g food = 31.3% (w/w)
% Cook: 24.9% / 31.3% x 100%	= 79.6%

ORDERING INFORMATION

YSI Part Numbers:

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) ■

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 hence to the glucose concentration.

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. 1N 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 mL of 1N acetate buffer.

G. Add 5 mL 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.

* The linear range of the system is 0.05–9.00 g/L glucose. If the value reported exceeds this, further dilution is required.

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.

VI. 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 glucose is produced when 1.0 g of starch is hydrolyzed, the glucose concentration of the sample should be multiplied by 0.9.

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

or

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

Example: 0.52 g of pet food was diluted to 100 mL in a Class A volumetric flask. The sample was prepared using the 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.

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%

ORDERING INFORMATION

YSI Part Numbers:

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) ■

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