

## Simultaneous Measurement of L-Lactate and Ethanol in Tomato-based Products

### I. 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 the YSI 2700 SELECT. YSI's unique enzyme electrode technology provides for specific lactate measurements in the range of 10 to 1335 ppm. 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 2787 buffer, the YSI 2700 analyzer simultaneously measures lactate and ethanol after aspiration of just 20 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 30 seconds after results and the YSI 2700 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.<sup>1</sup>

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 the YSI 2700 in process applications.

<sup>1</sup> 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.

### II. Materials and Setup

#### Sample Collection Guidelines

- Collection/mixing container (as specified by SOP)
- Transfer pipettes and related laboratory glassware
- Centrifuge (as specified by SOP)
- Collection tube to collect supernatant (as specified by SOP)
- YSI 2700D, configured for lactate and ethanol measurement

#### YSI 2700 Instrument Setup

See the YSI 2700 user's manual for general setup and safety information.

Chemistry electrode and reagent configuration requires a 2700D (two-electrode) system. The chemistry configuration is non-standard and will not be described in the 2700 user's manual. Other than the system buffer, all other configurations are described in the setup below. Use YSI 2787 system buffer to fill the buffer bottle.

Under Menu choose Setup, and then choose MeasParam to access the electrode assignment configuration. Set or confirm the following parameters.

Sample Size	20 microliters
Cal Method	Two Station
<b>Black Probe Parameters</b>	
Chemistry	Ethanol
Unit	mg/L (ppm)
Calibrator	1000 (YSI 2769)
End Point	30 seconds
CalStation#	2
<b>White Probe Parameters</b>	
Chemistry	Lactate
Unit	mg/L (ppm)
Calibrator	500 (YSI 2776)
End Point	30 seconds
CalStation#	1
<b>Autocal Parameters<sup>2</sup></b>	
Sample Error	ON
Temperature	1°C
Time	60 Min <sup>3</sup>
Sample	0 Sam
Cal Shift	2%
Calibration Station(s)	#1 and #2
Sample Station	#3 (or #4 if turntable)

<sup>2</sup> Enter AutoCal menu from RunMode under Setup Menu.

<sup>3</sup> Calibration is automatically updated hourly. To ensure best accuracy, manually update calibration (press Calibrate key) a few minutes prior to measuring a sample.



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### III. Method Guidelines<sup>4</sup>

- A. Calibrate the YSI 2700 by entering Run Mode or, if in Run Mode, by pressing the Calibrate key.
- B. Each day, prior to runs, perform the FCN membrane test using YSI 2363 solution.
- C. Each day, prior to runs, test the linearity of the system using YSI 2328 (1335 mg/L lactate) linearity standard.
- D. Each day, prior to runs, test the linearity of the system using YSI 2759 (500 mg/L ethanol) linearity standard.
- E. When idle for more than 15 minutes during sampling, initiate a calibration to ensure greatest accuracy.
- F. If measuring lactate and/or ethanol in your tomato product follow your company's SOP to test the change in lactate and/or ethanol concentrations in your sample.
- G. Compare changes to acceptance criteria related to flavor spoilage or other microbial contamination issues.

### IV. Results (Typical Precision & Accuracy)

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 2700 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 50ppm (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 100ppm 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 (mg/L) 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

<sup>4</sup> Steps A to E are standard for preparing the YSI 2700 each day. Steps F-G will be specific to the tomato product and application.

106.8 ppm and 128.0 ppm above base for lactate and ethanol, respectively.

#### YSI 2700 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 below for lactate and for ethanol.

##### 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%

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 2700. 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 below as the "Calculated" value. The value of the spiked sample obtained with the YSI 2700 is then designated the "Spiked" value.

Spiked samples of diluted ketchup were measured using the YSI 2700 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 the table below.

Sample	Spike*	Unspiked*	Spiked*	Calculated*	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

### V. Summary

Experimental results shown above demonstrate that the YSI 2700 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.

## VI. Ordering Information

YSI Part Numbers:

- 2700D 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
- 2787 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



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