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

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Abstract

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. In this study, Lactate and ethanol were measured in supernatants of diluted ketchup samples that were obtained from a commercially available source. Precision of replicate samples was determined from selected samples, and percent recovery was determined for samples spiked with both lactate and ethanol. Spiked samples of diluted ketchup were measured using the YSI Biochemistry Analyzer within 30 minutes of spiking and mixing. The results of this study demonstrated 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. This method of measuring lactate and/or ethanol can reduce QC test time by hours and provide results that better predict potential flavor issues and incipient spoilage compared to traditional microbial methods.

Analytical Method

Rationale:

• 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).

• Changes in lactate levels in cultures of approximately 100ppm can indicate incipient spoilage. 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.

Precision Analysis of Unspiked Samples:

• 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 centrifuge tubes and centrifuged ≥ 5 minutes.

• Sample supernatants were analyzed on a YSI Biochemistry Analyzer for ten consecutive measurements each of L-lactate and ethanol.

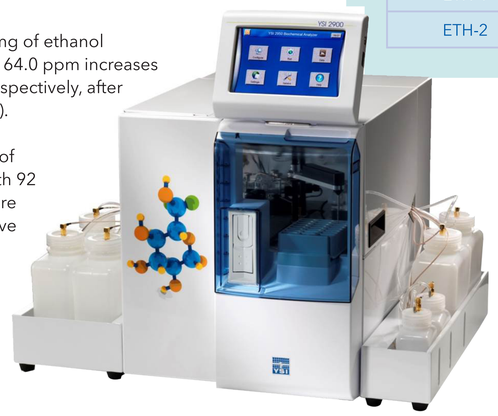
• Results were recorded and the precision of each analyte was determined. The final base lactate and ethanol readings were averaged and used to calculate spike/recovery values in the spiked sample study.

Spiked Sample Study:

• In a 100 ml volumetric cylinder, 2.0 ml of lactate standard and 2.0 ml of ethanol standard were combined with 96.0 ml of ketchup that had been diluted 1:1 with reagent water.

• Spike additions of 53.4 mg of lactate and 64.0 mg of ethanol represented theoretical changes of 53.4 ppm and 64.0 ppm increases above base lactate and ethanol concentrations, respectively, after volume correction (0.96 x unspiked concentration).

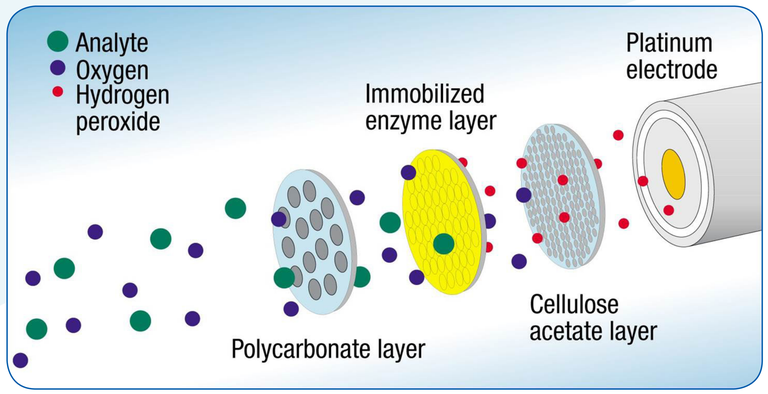
• Following the protocol described above, 4.0 ml of L-lactate and 4.0 ml of ethanol were combined with 92 ml of diluted ketchup. The theoretical changes were determined to be 106.8 ppm and 128.0 ppm above base for lactate and ethanol, respectively.



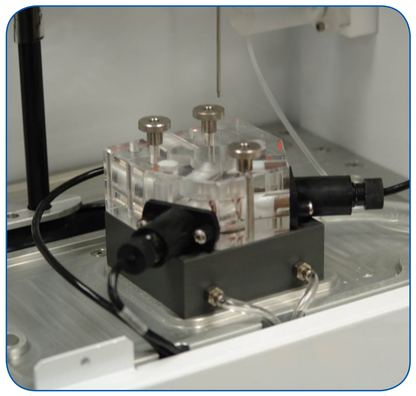
YSI 2950D
Biochemistry Analyzer

How the Immobilized Enzyme Biosensor Technology Works

Ketchup samples loaded onto YSI Analyzer with either microcentrifuge tubes or 96-well plates.



Exploded view of membrane and sensor.



Sample (15 µl volume) deposited into sample chamber configured with L-lactate oxidase and alcohol oxidase immobilized enzyme membranes.

$$\text{L-Lactate} + O_2 \xrightarrow{\text{L-Lac Oxidase}} H_2O_2 + \text{Pyruvate}$$

Simultaneous measurement of lactate & ethanol < 2 minutes.

$$\text{Ethanol} + O_2 \xrightarrow{\text{Alcohol Oxidase}} H_2O_2 + \text{Acetaldehyde}$$

L-Lactate/Ethanol Analysis of Diluted Ketchup

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

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

Analyzer Precision for Unspiked Samples

• Samples diluted 1:1

• Mean ppm values (n=10) shown for sample L-Lactate & Ethanol concentration

• Analytical precision (%CV) of diluted ketchup samples ≤ 2.25%

Spiked Samples of Diluted Ketchup

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%

Percent Recovery of Spiked Samples

• Spiked samples of diluted ketchup were measured using the YSI Biochemistry Analyzer within 30 minutes of spiking and mixing.

• Mean values (n=3) shown.

• Percent recovery of calculated spiked samples = 100.0 ± 4.1%

Conclusions

• L-lactate is useful in identifying growth of lactic acid bacteria while ethanol identifies the potential presence of yeasts and molds.

• Analytical method appropriate for screening potential flavor issues and incipient spoilage in finished product.

• Immobilized enzyme biosensor technology provides adequate precision and accuracy to make process and quality assurance decisions in tomato product manufacturing.

• YSI Analyzer accurately measured lactate and ethanol simultaneously in a tomato matrix within two minutes.

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