

Production and Determination of Ethanol Content in Samples Treated with Glucose and *Candida albicans*

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Abstract

Ethanol analysis is an important analysis often conducted in forensic science. *Candida albicans*, a common yeast found in human remains, has been shown to produce elevated levels of ethanol in post-mortem analysis, especially in subjects with diabetic or dietary sugar conditions. This research focuses on the investigation of ethanol produced by *Candida albicans* in samples, with a consideration of the potential glucose effect on production. The quantity of glucose present determines the amount of ethanol produced when *Candida albicans* is present to convert glucose into ethanol. It is assumed that the amount of glucose present is proportional to the amount of ethanol produced, and so, as glucose concentration increases, the ethanol levels increase accordingly. For this research, yeast samples were grown and some were exposed to varying levels of glucose in order to examine the correlation glucose has with ethanol production. Samples were analyzed with ultraviolet/visible spectroscopy, and the limit of detection and limit of quantification were determined. Ethanol was successfully detected and quantified in the yeast samples that were exposed to different glucose levels. The ultimate goal of this research is to quantify a relationship between glucose levels in human remains and elevated post-mortem ethanol concentrations.

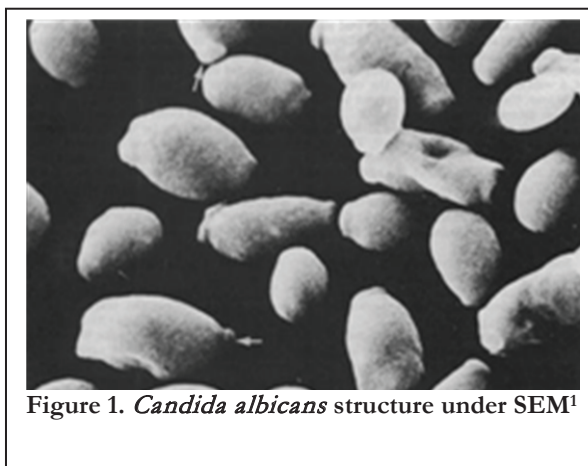


Figure 1. *Candida albicans* structure under SEM¹

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Candida albicans and glucose ($C_6H_{12}O_6$) are substances capable of increasing the content of ethanol in certain samples.² *Candida albicans* is a common type of yeast that

can frequently be found in the environment.² It has been shown that *Candida albicans* can increase the content of ethanol in postmortem blood samples, especially in samples that contain elevated C₆H₁₂O₆ conditions.² It is assumed that C₆H₁₂O₆ is a type of sugar that *Candida albicans* can use in order to increase the production of ethanol.² To investigate the production of ethanol, a dichromate-ethanol reaction commonly used in breathalyzer tests was utilized. The dichromate-ethanol solution was exposed to varying amounts of glucose within the *Candida albicans*. The exposed solutions were analyzed using spectroscopic analysis. It is assumed that higher amounts of glucose lead to lower absorbance readings, which indicate higher percentage ethanol content, thus indicating that higher amounts of C₆H₁₂O₆ lead to an increase in ethanol production supported by the *Candida albicans*.²

Methodology

Materials and Methods

Absolute ethanol was purchased from Sigma Aldrich. Potassium dichromate was purchased from J.T. Baker. Silver nitrate, sulfuric acid, and glucose were purchased from Fisher Scientific. The dichromate solution stock was prepared by mixing 0.0500 g K₂Cr₂O₇, 0.0500 g AgNO₃, 100.00 mL D.I. H₂O, and 100.00 mL of concentrated H₂SO₄. The blank solution was prepared by mixing 5.00 mL concentrated H₂SO₄ and 5.00 D.I. H₂O. The stock ethanol solution was prepared by mixing 31 μ L absolute C₆H₅OH and 1000 mL D.I. H₂O. Solutions of EtOH content were made for 0.00, 0.025, 0.050, and 0.100% blood alcohol content (BAC) from the stock ethanol solution. All the solutions were filtered prior to analysis.

Candida albicans and Glucose Sample Preparation

The yeast was initially transferred to a Sabouraud Dextrose Agar (SAB), grown overnight for a 24-hour period at 32 °C. After 24 hours, the samples were stored in a refrigerator, which slows the growth of the yeast. The nutrient broth samples were autoclaved, and varying amounts of glucose (0.00 mg as a control, 1.00 mg, 10.00 mg, 50.00 mg, and 100.00 mg) were added. These samples were then inoculated using the aseptic technique and placed into the incubator for a 24-hour growth period. *Candida albicans* was provided by Carolina Biological.

UV/Vis Spectroscopy

Samples were measured using ultraviolet/visible (UV/V) spectroscopy. All spectroscopic measurements were obtained using an Ocean Optics Red Tide 650 UV/Vis Spectrometer with a 1 cm path length and the corresponding SpectraSuite Software, or a Genesys 20 Spectrometer. Absorbance readings were taken at λ_{max} of 440 nm.²

Analysis

In order to develop the dichromate solution for detection, potassium dichromate was mixed with an equal mass of silver nitrate, and the solution was diluted by using concentrated sulfuric acid and water.² The dichromate solution was exposed to varying amounts of ethanol standards and to *Candida albicans* samples with varying amounts of glucose. Any ethanol present in either the standards or the yeast samples

with glucose would result in a colorimetric change measured by a corresponding decrease in absorbance.

This colorimetric reaction is based on the breathalyzer reaction between ethanol and dichromate in acidic conditions, as given here.

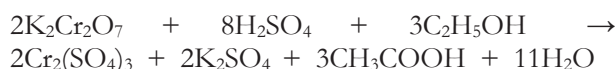


Figure 2. The reaction of Cr⁺⁶ being reduced to Cr⁺³ upon reaction with C₂H₅OH in acid²

Results and Discussion

In order to determine the optimal wavelength to be used for absorbance measurements, the dichromate samples were analyzed by using a spectrum seen in Figure 3.

The most reliable λ_{max} for detection is 440 nm, which we used in our research.² Figure 4 shows the change in color that occurs once ethanol has been added to dichromate. The color change is proportional to the concentration of ethanol.

The ethanol present is oxidized to acetic acid, and dichromate is reduced from Cr(VI) to Cr(III), resulting in a more colorless solution.² The ethanol content produced by the reaction of varying mg of glucose with *Candida albicans* were measured in percentage BAC in correlation with the measured absorbance. The inverse yet linear relationship in which absorbance decreases with an increasing amount of ethanol is shown in Figure 5 on the next page. A pattern of linearity can be seen from the graph, as it shows a correlation factor R² of 0.995. The higher the ethanol content of the standard, the smaller the absorbance readings will be. A limit of detection of 0.0042% BAC and a limit of quantification of 0.0132% BAC were established for this analysis method. This calibration curve was used

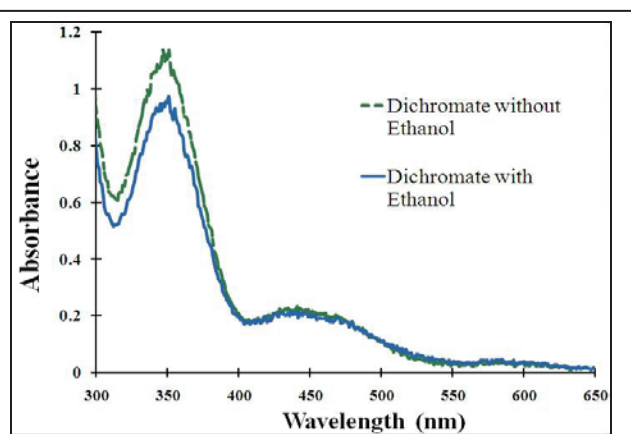


Figure 3. Absorbance spectrum of dichromate solutions with and without the addition of ethanol with a $\lambda_{\text{max}} = 440$ nm



Figure 4. The sample on left is the dichromate solution without ethanol, and sample on right is the dichromate solution with ethanol.

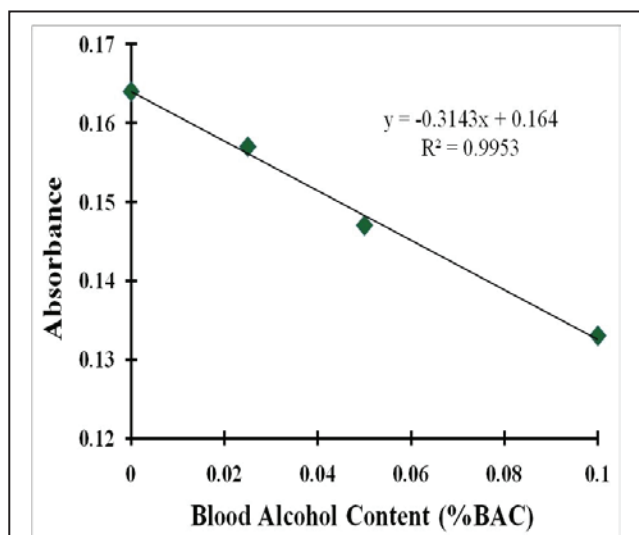


Figure 5. Calibration curve of % BAC vs. Abs. for ethanol standards reacting with the dichromate solution

Mass of C ₆ H ₁₂ O ₆ (mg)	% BAC
Control	0.079
1.00	0.115
10.00	0.200
50.00	0.245
100.00	0.283

Table 1. Percentage BAC values (EtOH content) obtained from *Candida* samples treated with varying amounts of C₆H₁₂O₆

to successfully determine the percentage BAC (EtOH content) in samples exposed to varying amounts of glucose within the yeast *Candida albicans*. We were able to quantify a relationship between the amount of added glucose, the corresponding absorbance readings, and the amount of ethanol produced by the yeast. The data in Table 1 shows how increasing the amounts of glucose in the *Candida* samples led to a proportional increase in the amount of ethanol (percentage BAC) quantified.

The more glucose that was added, the lower the absorbance readings, indicating a higher content of ethanol for each sample. An increase in percentage BAC shows that *Candida albicans* is capable of producing ethanol from glucose. By using this information, we are able to determine a quantitative relationship between glucose and ethanol production. This is an important fact regarding ethanol production, and it can be used for determining percentage BAC in blood samples, especially in those samples where blood contains excess levels of glucose, such as the blood of diabetic patients. This connection between glucose levels and

ethanol levels is especially important to consider in post-mortem samples in which high percentage BAC levels have been reported. A high percentage BAC can yield a false implication in toxicology and police reports.²

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