STUDIES ABOUT WINE FERMENTATION USING OPTICAL FIBRE BIOSENSOR

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Abstract

It was studied the ethanol concentration in wine fermentation with and without added yeast using an optical fiber biosensor made by ADH immobilization onto optical fiber by sol-gel technique in MTMOS/TEOS matrix.

Key words: optical fibre, biosensor, wine, fermentation

INTRODUCTION

It were studied the ethanol concentrations in wine fermentation with and without added yeast using optical fiber sensor made by ADH immobilization onto optical fiber by sol-gel technique in MTMOS/TEOS matrix.

MATERIAL AND METHODS

Materials

All materials used for determinations were purchased from Sigma-Aldrich Company (Germany).

- β-nicotinamide-adeninedinucleotide (NAD+) solution 0,063 mM;
- Alcohol dehydrogenase enzyme ADH (EC 1.1.1.1) 60 U/ml;
- Ethanol absolute (99, 95 %);
- Tetraethoxysilane 98% (TEOS);
- Methyltrimethoxysilane 98% (MTMOS);
- Solution HCl 1N;
- Solution NH_4OH 1N;
- Phosphate buffer solution pH = 7;

Equipment

For the experiments the following equipment were used:

- Portable laser spectrophotometer, Jazz from Ocean Optics, with two channels, one channel of spectrometric type "master" for UV-VIS range (1025-200 nm) and a "slave" channel for the VIS-NIR (360-1100 nm). The spectrophotometer is equipped with a built-in microprocessor and OLED display for independent operation (without PC), with 2-channel and source excitation deuterium-halogen-tungsten, 210-1100 nm, 7W, with integrated power supply lithium-ion battery, included in the platform.
- Optical Fiber Bifurcated - for the 210-1100 nm, consisting of 2 fibers, one for enlightenment, the other for reading, fiber length 2 m, SMA 905 connectors;
- SMA 905 fiber Multimode connector, prepared for enzyme deposition: the cutting end of the optical fiber was polished and a portion of 30-40 mm of the cladding has been removed for the next enzyme applying steps.
- Software Spectrasuite for acquisition and real time analysis of data, as well as for data processing.
- UV-VIS Spectrophotometer Specord 210 Plus (Analytic Jena, Germany).

Method

The developed sensor is based on a plastic optical fiber with the core diameter of 1mm terminated with a sensing element. The fiber end was cut, polished and a portion of 30-40 mm of the cladding has been removed for the next enzyme applying steps. The sensing matrix is made from MTMOS/TEOS hybrids obtained from sol-gel technique.

Basic working principle is based on a classic colorimetric approach. The interrogator light is scattered and absorbed by the sensing matrix that include ADH and NAD^+. The NADH produced in biochemical reaction induces a change of the optical spectrum of the outgoing light collected by the fiber. Analyzing the spectrum of this optical signal the concentration of the ethanol can be measured.

The technique involved the sol-gel enzyme immobilization under mild conditions of temperature and pressure without altering the biological activity. A certain amount of MTMOS:TEOS was mixed by magnetic stirring in a glass bottle in the ratio 1:1 with solution HCl 1N and the mixture was homogenized for 2-3 hours and then the pH was adjusted to 6 with ammonium hydroxide solution. It was added the NAD^+ solution and ADH solution and the fiber optic was immersed into it and stirred it with a certain speed assuring a uniform film matrix deposition onto optical fiber. The deposited layer was dried at room temperature for 24 hours prior to use and kept immersed in phosphate buffer pH=7. The measurements were performed at room temperature.

To evaluate the performance of the developed sensor, the spectral properties of the sensor have been measured using Spectrasuite software for data acquisition and analysis in real time. The results were compared with spectral analysis realized on the UV-VIS spectrophotometer Specord 210 plus (Analytik Jena, Germany) performed after the standard spectrophotometric enzyme assay [6, 7].

In order to test the optical fiber sensor and to determine the alcohol content the standard curve was set up, using ethanol solutions in the concentration range of 2% to 18 %, as presented in figure 1. The sensor was immersed in ethanol solutions and the optical density at 340 nm was determined. This figure shows a linear relationship which can be used in quantification of ethanol concentration.

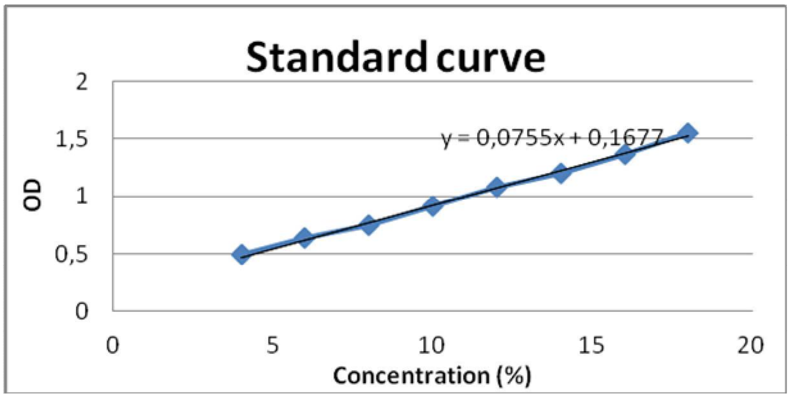

Fig 1. Standard curve

RESULTS AND DISCUSSIONS

In order to test the applicability of the sensors to beverages, the biosensors were tested on wine samples obtained from alcoholic fermentation of the grape must realized at Bay Zoltan Institute of Biotechnology from Szeged, Hungary and the results were compared with the standard spectrophotometric enzyme assay [6,7]. It is well observed the maximum of absorbance at 340 nm corresponding to NADH obtained in fermentation reaction for ethanol detection.

Wine production

The grape samples were physically crushed to make must by a fruit press (Green Star GS- 1000) and frozen must was taken into two fermenters, which were incubated at 21±2°C. One of them was completed with commercial wine yeast (20g/hl). The systems were mixed with 100 rpm, the pH was 4.2-4.3, the dissolved oxygen reached 0% after start. Liquid samples were taken for analysis of ethanol content.

The absorbance spectra of white wine and red wine sample with and without yeast achieved by optical fiber sensors are presented in figure 2, 3, 4 and figure 5.

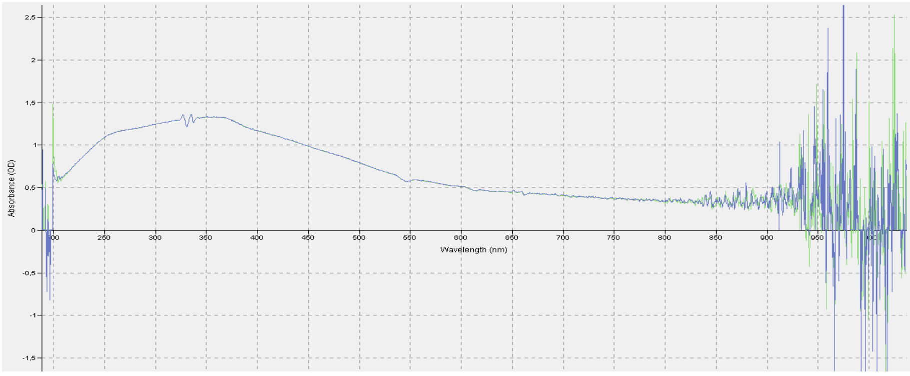

Fig. 2. Absorbance spectra of white wine sample with yeast achieved by optical fiber sensor

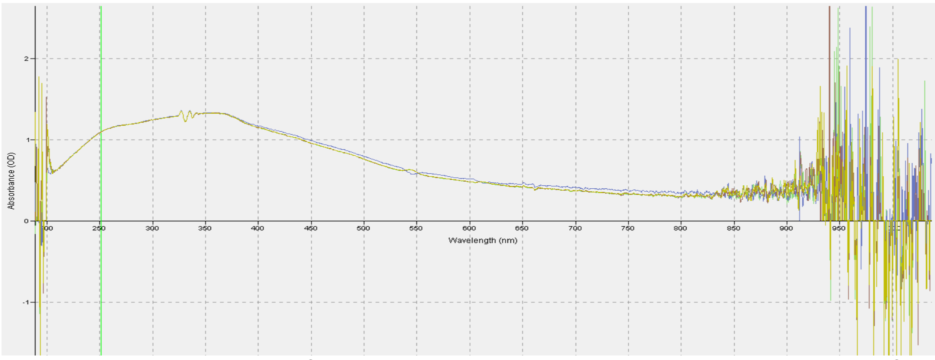

Fig. 3. Absorbance spectra of white wine sample without yeast achieved by optical fiber

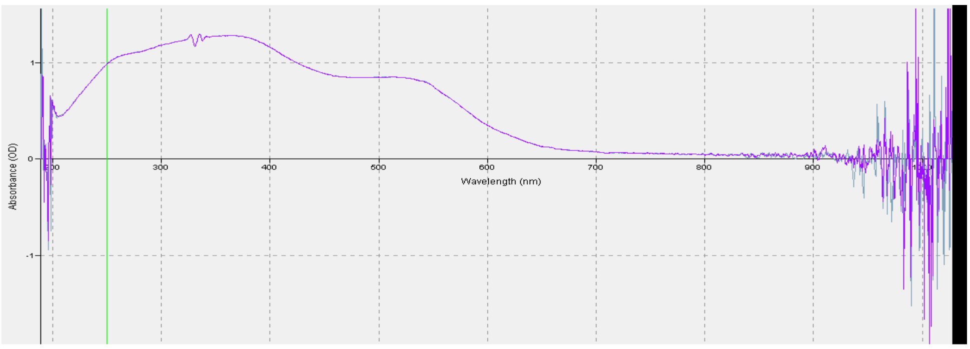

Fig. 4. Absorbance spectra of red wine sample with yeast achieved by optical fiber sensor

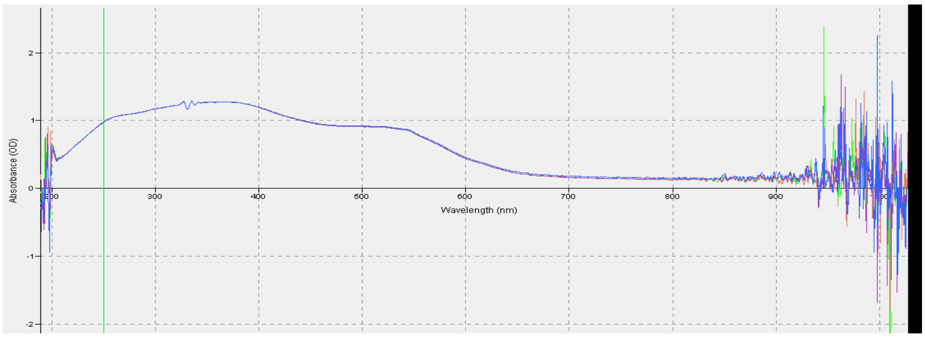

Fig. 5. Absorbance spectra of red wine sample without yeast achieved by optical fiber

All the spectra show a well visible maximum at the wavelength at about 340 nm These results indicate the possibility for a good application of the optical fiber biosensor for ethanol monitoring in grape must during the fermentation process as well as in wines and other products.

The ethanol content in white and red wine during the fermentation with or without added yeast is presented in *Table 1*.

Table 1

Ethanol content in white and red wine during the wine fermentation measured spectrophotometric

Wine Samples	**Ethanol concentration measured using optical fiber biosensor (%)**	**Ethanol concentration measured using standard spectrophotometric enzyme assay (%)**
Red wine	8.74 ± 0.01	8.81 ± 0.004
Red wine with added yeast	9.44 ± 0.02	9.48 ± 0.003
White wine	9.34 ± 0.01	9.28 ± 0.003
White wine with added yeast	9.75 ± 0.03	9.73 ± 0.001

CONCLUSIONS

The new developed optical fiber biosensor obtained by simultaneous immobilization of alcohol dehydrogenase (ADH) and NAD+ onto optical fiber by sol-gel technique in MTMOS/TEOS matrix is suitable for the ethanol concentration determination for the range of 2-18% ethanol and it allows experimental determinations in real time. Adding yeast in the wine fermentation determined an increasing of wine ethanol content.

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REFERENCES

1. Ahuja T., Mir I. A., Kumar D., Rajesh K., 2007, Biomaterials, 28, 5, p.791–805.
2. Aubonnet S., Barry H. F., Von Bueltzingsloewen C., Sabbatie J. M., Maccraith B. D., 2003, Electron. Lett., 39, no.12, p.913-914.
3. Azevedo A. M., Miguel F., Joaquin M. S., Fonseca L. P., 2005, Biosensors and Bioelectronics, 21, p.235-247.
4. Balat M, Balat H., 2009, Apll. Energ, 86, p.2273-2282.
5. Baldin I., F., Giannetti A., Mencaglia A., Trono A. C., 2008, Curr. Anal. Chem., 4, , p.378-390
6. Bernt E., Gutman I., 1974, Methods in enzymatic Analysis, 3 rd edn, Edited by H. U. Bergmeyer, Academic press, New York, 3, , p.1499-1502.
7. Beutler H., 1984, Methods of Enzymatic Analysis, 3rd edition, VCH, Weinheim-Deerfield Beach, FL-Basel 6, p.598-606.
8. Bhatia R. B., Brinker, 2000, C. J., Chem. Mater, 12p.2434-2441.
9. Cagnol F., Grosso D., Sanchez C., 2004, Chem. Commun, p.1742-1743.
10. Fabbri P., LeonellI C., Messori M., Pilati F., Toselli M., Veronesi P., Morlat-Therias S., Rivaton A., Gardette J. L., 2008, J. Appl. Polym. Sci., 108, no. 3, p.1426-1436.
11. Fabbri P., Pilati F., Rovati L., Mckenzie R., Mijovic J., Opt. Mater., 2011, 33, no.8, p.1362-1369.
12. Lin J., Brown C.W., 1997, Trac-Trends Anal. Chem., 16, no.4, p.200-211.
13. Messori M., Toselli M., Pilati F., Fabbri E., Fabbri P., Busoli S., 2004, Surf. Coat. Int., 86, no. 3, p.183-186.
14. Sanchez C., Shea K. J., Kitagawa S., 2011, Chem. Soc. Rev., 40, p.471-472.
15. Toselli M., Marini M., Fabbri P., Messori M., Pilati F., 2007, J. Sol-Gel Sci. Technol., 43, no. 1, p.521-533.
16. Wen J., Wilkes G. L., 1996, Chem. Mater., 8, no.8, p.1667-1681.