JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Ethyl Alcohol Content in Red Wines with an Optical Alcohol Meter Based on Nanostructured Silicon

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A rapid analytical method for selectively monitoring ethyl alcohol in red wines without sample pretreatment, based on the use of an optical sensor, has been developed exploiting a porous silicon microcavity. The optical structure, realized by alternating layers of different porosities, was stabilized by thermal oxidation, and the resonant peak shift of the microcavity (projected at 600 nm) was monitored in the presence of more than 20 red wines. The resonant peak shows an increasing red shift depending on the wine alcoholic strength, which is ascribable to a change of refractive index due to physisorption/condensation of ethanol vapors inside the pores' structure. The linear response of the PS oxidized microcavity to the wine alcoholic strength insures the determination of the ethanol amount with a high accuracy and reliability. The calculated values differ by less than 0.5% to those obtained with the official method in accordance with the limits imposed by European laws. Moreover, a user-friendly interface, allowing the sensor to be used by unskilled persons, and portable packaging, able to ensure in situ measurements, have been developed.

KEYWORDS: Optical sensor; ethanol; PSOM; microcavity; porous silicon; wine

INTRODUCTION

Growing high-quality requirements and an increasing safety demand are currently two of the most important goals of the food industry; indeed, in recent years, there has been an increasing request of rapid, selective, and low-cost sensor devices able to ensure the analysis of target molecules in complex biological matrixes, as foods are. In this context, an important task is the quantitative determination of ethyl alcohol since the production of alcoholic beverages such as wines, beers, and liquors plays a significant role in the present-day food industry. Moreover, ethanol is a key parameter in fermentation processes, and it is a quality indicator for spirits, beers, and wines.

Nowadays, the official methods to quantitatively determine the wine alcoholic strength (AS) are given by the International Organization of Vine and Wine (OIV) (*1*) and by the European Commission (*2*); usually, they require a first distillation step, to be performed following a strict protocol, and a second step of a density measurement (using a picnometer, an electronic frequency oscillator, or a hydrostatic balance) or refractive index measurement of the distillate. The AS also can be obtained by means of other methods, certified by the Association of Analytical Chemistry (AOAC) (*3*) including gravimetric techniques and gas/liquid chromatographic analysis (*4*−*6*).

Notwithstanding the high performance of the mentioned methods, they are not able to satisfy the needs of the drink industry in terms of express analysis, automation, and inexpensiveness since they are time-consuming, require sample pretreatment, and in some case necessitate professional laboratories with expensive equipment and specialized personnel. Moreover, they do not allow continuous monitoring during industrial processes and are not applicable for routine analysis. In the wine production, these aspects are of extreme importance since the winemakers need to monitor the ethanol amount in the must, throughout the process of fermentation, as well as in the final product.

To date, different approaches have been proposed to face this problem such as electrochemical biosensors (*7*−*12*) and optical sensors (*13*). The electrochemical biosensors are commonly based on the amperometric response of standard electrodes modified with enzymes, alcohol oxidase (AOX) (*7*−*10*), or alcohol dehydrogenase (ADH) (*11*, *12*) and measure the current generated by oxygen consumption or by electrooxidation of species arising from the selective conversion of ethanol. The optical sensor correlates the color change of the Reichardt phenolbetaine dye, dissolved in a plasticized copolymer, to the ethanol content of hydroalcoholic solutions (*13*). Both the sensors ensure a good selectivity and sensitivity, but their main drawbacks are the low stability and short lifetime: the biosensors' performance, in the best case (*11*), allow measurements for 90 days but only if they are kept in a refrigerator in a dried form; the optical sensor, due to dye photobleaching, begins to

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10.1021/jf070809w CCC: $37.00
Published on Web 07/04/2007

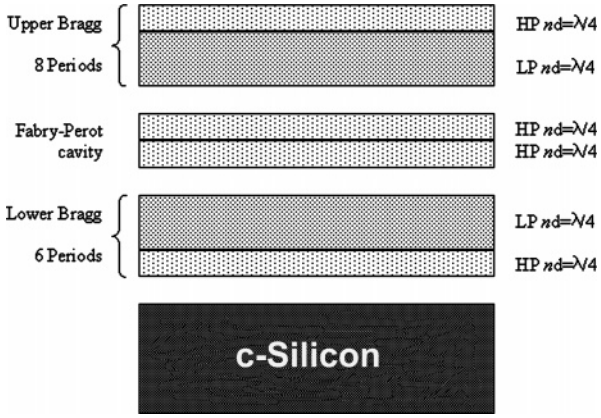

Figure 1. Scheme of the PSOM structure.

lose its detection capabilities after 1 day under continuous measurements.

This paper describes an optical sensor based on a porous silicon (PS) microcavity, projected in the visible range, which allows the measurement of the wine AS with a high accuracy, sensitivity, and reliability overcoming the bio/optical sensors' drawbacks. PS-based optical devices have been extensively exploited for sensing applications by several groups (*14*−*17*), and also the authors have already used a PS oxide microcavity (PSOM) as an ethanol sensor (*18*), but hereinafter a new setup, obtained by integrating the PSOM in a portable device (allowing in situ measurements and its use by unskilled persons), and different working conditions will be proposed.

Moreover, the selectivity and sensitivity of the PSOM will be compared to two analytical methods widely used for monitoring the wine AS: (i) the official method, described in ref *1*, based on wine distillation and density measurements of the distillate and (ii) the Fourier transform infrared method based on the analysis of characteristic absorption bands, due to ethyl alcohol, in the mid-infrared range.

MATERIALS AND METHODS

PSOM Fabrication. The PS microcavity was made of two distributed Bragg reflectors (DBRs) alternated by a Fabry−Perot resonator (**Figure 1**) and was produced through anodic etching of a single crystalline silicon wafer obtained from MEMC Electronic Materials (Novara, Italy), <100> oriented, highly doped with boron atoms (resistivity 8−12 mΩ cm). The etching solution was a 1:1 mixture of hydrofluoric acid (50% in water) and ethyl alcohol (>99.9%), and both were obtained from Carlo Erba reagents (Milan, Italy).

The DBRs were made of periods, eight for the upper and six for the lower, of two alternating layers, obtained by applying two current density values (150 and 450 mA/cm^2). These values allowed the modulation of the porosity (150 mA/cm^2 low porosity (LP) −to 450 mA/cm^2 high porosity (HP)) and hence of the refractive index of the material. The Fabry−Perot resonator was made of one single period composed of two HP layers. Each single layer had an optical thickness *nd* of $\lambda/4$, where *d* is the physical thickness, *n* is the refractive index, and λ is the Bragg wavelength ($\lambda = 600$ nm) at which the microcavity has been projected.

The as-prepared microcavity was oxidized, due to the instability of the pristine material, and the oxidation was carried out by means of thermal treatment, using an ASM oxidation furnace, through the following receipt: (i) pre-oxidation at 400 °C for 1 h in O_2 flow, 2 L/min; (ii) ramp from 400 to 850 °C in N_2 flow, 1 L/min; (iii) oxidation at 850 °C for 30 min in O_2 flow, 5 L/min; and (iv) ramp from 850 to 300 °C in N_2 flow, 1 L/min.

The refractive index of the as-prepared as well as the oxidized layers was estimated by means of a simulation program: SCOUT (*19*). The simulations were carried out using an effective medium approximation (EMA) based on the Bruggeman approach; the dielectric constants of vacuum and silicon dioxide were used in the EMA. The resulting PSOM has a fwhm around 15 nm.

Table 1. Red Wines Used for Calibration[a]

		AS (% volume)	
code	wine	OIV method	FTIR method
W1	Dolcetto d'Alba DOC	11.07	11.10 ± 0.0058
W2	Barbera d'Alba DOC	12.47	12.52 ± 0.0153
W3	Barbera d'Alba DOC	12.64	12.57 ± 0.0153
W4	Barbera d'Alba DOC	12.92	13.00 ± 0
W5	Barbera d'Alba DOC	13.01	12.96 ± 0.0153
W6	Barbera d'Alba DOC	13.04	13.01 ± 0.0058
W7	Barbera d'Alba DOC	13.13	13.13 ± 0.0058
W8	Nebbiolo d'Alba DOC	13.26	13.31 ± 0.01
W9	Nebbiolo d'Alba DOC	13.40	13.31 ± 0
W10	Barbera d'Alba DOC	13.55	13.54 ± 0.0115
W11	Nebbiolo d'Alba DOC	13.79	13.69 ± 0.0058
W12	Barbera d'Alba DOC	13.93	13.95 ± 0
W13	Barolo DOCG	14.12	14.02 ± 0.0058
W14	Nebbiolo d'Alba DOC	14.36	14.35 ± 0.0058
W15	Nebbiolo d'Alba DOC	14.67	14.55 ± 0
W16	Barolo DOCG	14.84	14.73 ± 0.01
W17	Rosso da Tavola	15.01	14.88 ± 0.01

[a] Values in OIV column were used in **Figure 6**.

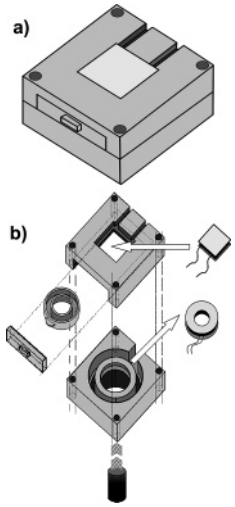

Figure 2. Scheme of the alcohol meter: (**a**) compact view and (**b**) deconstructed view.

Wine Samples and Analytical Methods. Two series of red Italian wines produced in Piedmont (northwest Italy) have been measured to verify the PSOM behavior. The first 17 samples were used to prepare a calibration curve (**Table 1**), and the second four samples were used to verify the calibration and to test the sensor response.

The AS of all the wines was determined using the two analytical methods. In the first, the wines were distilled volume by volume, and the density of the distillates was measured according to the OIV method (*1*) and the European Regulation (*2*). In the second, a WineScan FT120 Basic (FOSS Analytical A/S, Hillerød, Denmark) employing a purpose built Fourier transform infrared spectroscopy interferometer (FTIR) was used. Each sample was analyzed once for the OIV method and twice for the FTIR method.

Alcohol Meter Design. The alcohol meter is made of two main parts: (i) a Plexiglas cell, depicted in **Figure 2** (compact and deconstructed view), where the PSOM and the wines and/or the solutions are positioned and (ii) a measurement apparatus to monitor the shift of the PSOM, a high-resolution (HR2000) UV−vis fiberoptic spectrophotometer (from Ocean Optic Inc., Dunedin, FL).

The bottom and top part of the Plexiglas cell are shaped to allow the housing of two Peltier thermoelectric coolers, which permit us to tune the temperature from 15 to 50 °C. The bottom Peltier is perforated,

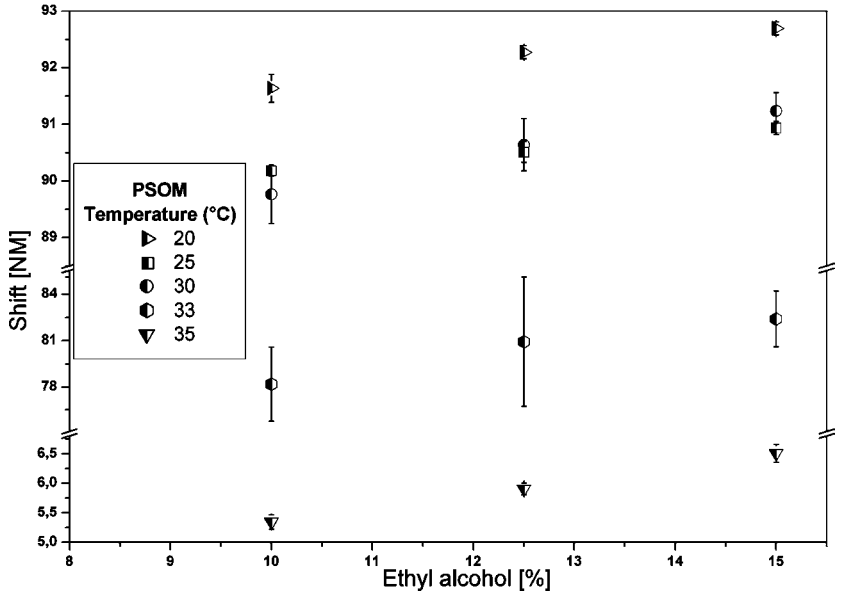

Figure 3. Shift of the PSOM at different temperatures as a function of the ethyl alcohol concentration in reference water/ethanol solutions (five measures each). Temperature of the solutions: 38 °C.

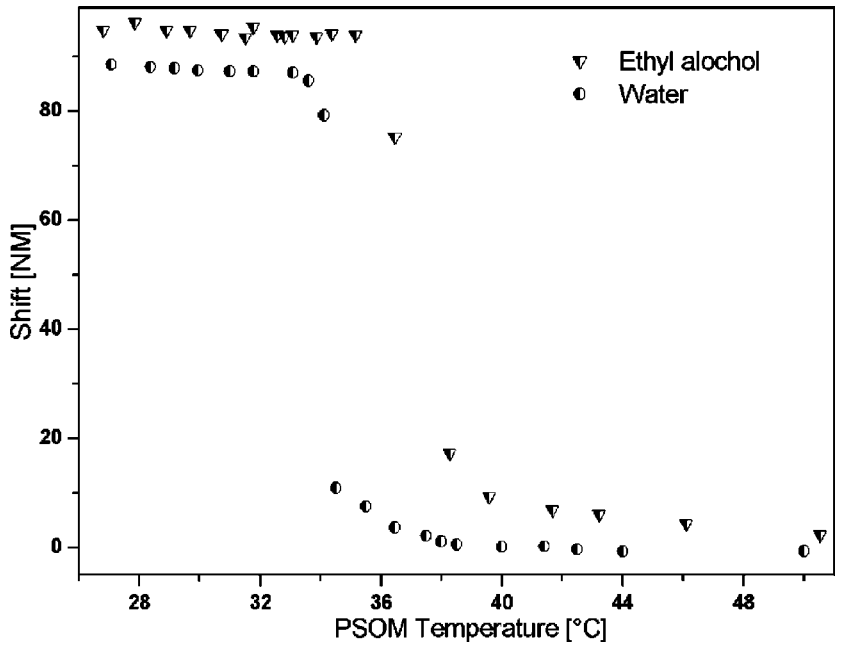

Figure 4. Dynamic shift of the PSOM as a function of the temperature for two different solutions: water and ethyl alcohol (≥99.9%). Both the solutions were kept at 38 °C.

and a bifurcated optical fiber is inserted into the hole to bring the light from the source (HL 2000 tungsten halogen lamp, Ocean Optics Inc.) as well as to collect the light reflected back by the PSOM. The PSOM is glued with a thermal paste on the upper Peltier and faces the fiber. The wine container, a few milliliters in capacity, has a circular form (same dimension and shape as the bottom Peltier) and is made of aluminum to ensure a fast heat exchange.

Alcohol Meter Procedure. The procedure to evaluate the wine AS, since it does not necessitate sample pretreatment, is extremely simple and requires the following steps: (i) pour 2 mL of the unknown wine in the aluminum container; (ii) read the position of the PSOM (≈600 nm); (iii) insert the container inside the Plexiglas cell, close it, and wait for equilibrium (the PSOM will shift of a variable quantity depending on the amount of vapors physisorbed/condensed inside the pore structure); (iv) read the equilibrium position reached by the PSOM (600 nm + X nm); and (v) remove the container and allow the PSOM to recover the original position (600 nm). The linear correlation between the shift of the PSOM (X nm) and the ethyl alcohol amount will allow the measurement of the wine alcoholic concentration.

RESULTS AND DISCUSSION

Alcohol Meter Optimizations. The temperatures of the wines as well as of the sensing element (PSOM) are the two key parameters to control the alcohol meter performance since they regulate the concentration of the volatile compounds in the gas phase and inside the pores structure, and therefore, they influence the equilibrium and the recovery time. To optimize them, the temperature of the bottom Peltier is kept at 38 °C (temperature selected considering previous results (*18*)), and the PSOM temperature is varied from 20 to 35 °C in a discontinuous way in five steps: 20 to 25 to 30 to 33 to 35 °C. At each temperature, three reference solutions of ethyl alcohol (≥99.9%) in deionized water at different concentrations (10 to 12.5 to 15%) are measured, 5 times each, and the PSOM shift is analyzed (**Figure 3**).

Low temperatures (≤30 °C) cause a large overall shift (**Figure 3**), more than 90 nm, but small relative shifts, the

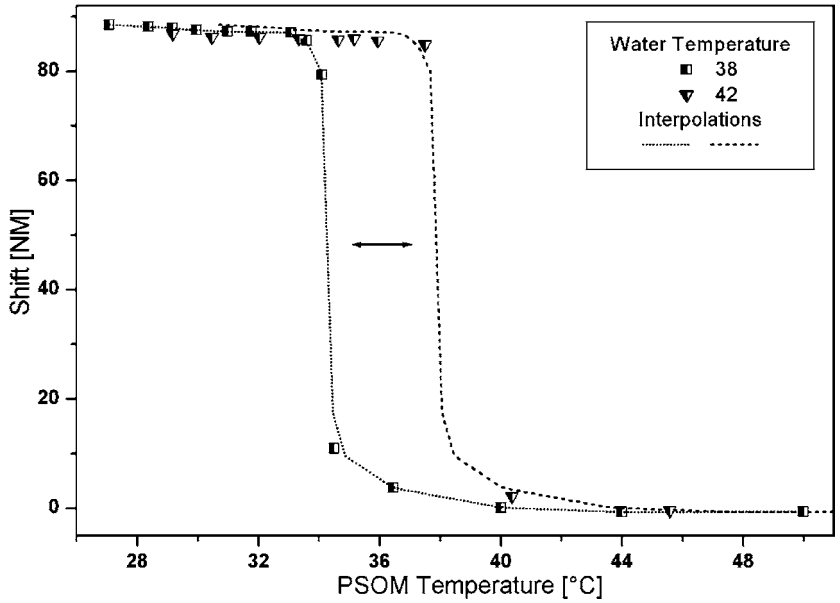

Figure 5. Dynamic shift of the PSOM as a function of the temperature for a water solution kept at 38 and 42 °C.

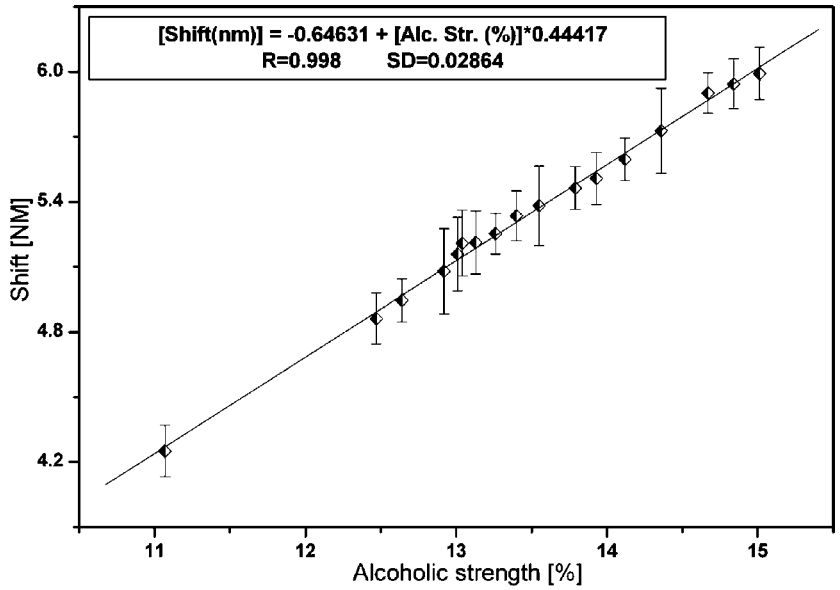

Figure 6. Calibration curve obtained measuring the wines listed in **Table 1** (five measures each).

difference between consecutive concentrations (such as 10 and 12.5%), which are below 1 nm and comparable to the error bars. In increasing the temperature to 33 °C, we observed a slight decrease of the overall shift and an improvement of the relative one, but unfortunately, the error bars enormously increased (around 2−3 nm). The best results are observed at 35 °C where, notwithstanding the low overall shift (5−6 nm), the difference between 10 and 15% is around 2 nm and the error bars are below 0.1 nm. A further advantage of this temperature is the low recovery time of the PSOM: a measure requires seconds instead of minutes for 20, 25, and 30 °C.

To clarify these behaviors, the PSOM shift is monitored with increasing the temperature continuously from 28 to 50 °C. **Figure 4** shows a steep decrease of the shift (from 90 to 10 nm) around 32 or 35 °C for both the reference solutions analyzed: water and ethyl alcohol, respectively. This sudden decrease allows us to understand the wide shift difference between the low (≤30 °C) and the high (35 °C) temperatures and, moreover, allows us to explain the large error bars observed at 33 °C, the temperature closest to the step. The shape of this curve appears to be similar to an adsorption isotherm, and the step could be assigned to a transition from a multilayer regime (condensation state, the PSOM pores are completely filled by water/ethanol) to a monolayer regime (physisorption state). This adsorption isotherm-like behavior could be explained if the adsorptive molecules have a greater affinity for one another than they do for the PSOM, but a detailed explanation of this phenomenon exceeds the aim of this paper and will not be proposed.

The other parameter to know, to optimize the performance, is the temperature of the solution and/or wine. To reach this temperature goal, the dynamic shift of the PSOM, increasing the temperature from 28 to 50 °C as shown in **Figure 4**, is monitored by maintaining the water solution at 42 °C instead of 38 °C; the results are shown in **Figure 5**. The PSOM shift is dependent on the temperature of the solution, but notwithstanding, the steep decrease is displaced to high values, increasing the temperature of the water solution, and it keeps the same shape. Considering all the optimization steps, the alcohol meter

Table 2. PSOM Shift (Mean Value and Error Bars) and AS of the Four Unknown Red Wines[a]

code	wine	shift (nm)	AS (% vol)
W18	Dolcetto d'Alba DOC	4.728 ± 0.09391	12.0997 ± 0.2046
W19	Rosso da Tavola	5.252 ± 0.09391	13.2794 ± 0.2047
W20	Barbera d'Alba DOC	5.378 ± 0.09391	13.5631 ± 0.2047
W21	Barbera d'Alba DOC	5.772 ± 0.1205	14.4501 ± 0.2340

[a] Wine AS are calculated using the calibration.

has been tested maintaining the PSOM at 35 °C and the bottom Peltier at 38 °C.

Alcohol Meter Performance. Figure 6 shows the calibration curve obtained by measuring the wines listed in **Table 1** using the alcohol meter procedure described previously; the AS values reported on the abscissa are those obtained by means of the OIV method (*1*, *2*). The optimized parameters (35 °C for the PSOM and 38 °C for the wine) allow making a single measure in 3 min including the PSOM recovery time. Each wine has been measured 5 times and has required 15 min.

The PSOM shift follows a linear behavior increasing the AS, as expected from the literature (*17*), and this linearity allows supposing a negligible contribution of the other volatile compounds of wine to the overall shift. This assumption is reasonable considering that acetic acid has a concentration in the vapor phase 3 orders of magnitude lower than ethyl alcohol, notwithstanding that it is the second most concentrated volatile compound in wine (concentration in the liquid phase ≤1g/L). The other volatile compounds have concentrations on the order of μg/L, and they cannot influence the shift.

The equation of the straight line, derived from the linear fit of the data reported in **Figure 6**, will be the tool to calculate the AS of the unknown red wines. The parameters *A* and *B* and the standard deviation (SD) were estimated with the least-squares method

$$A = \bar{Y} - B\bar{X},\ B = \frac{\sum_i^n (X_i - \bar{X})(Y_i - \bar{Y})}{\sum_i^n (X_i - \bar{X})^2},\ \mathrm{SD} = \sqrt{\frac{\sum_{i=1}^n (y_i - (A + Bx_i))^2}{N - 2}}$$

Table 2 reports the names of the four wines to be analyzed, the PSOM shift, and the AS values calculated using the following equation:

$$[\mathrm{AS}\ (\%)] = \{[\mathrm{shift}\ (\mathrm{nm}) - a]/b\};\ a = -0.64631 \pm 0.09822;\ b = 0.44417 \pm 0.00727$$

The AS variation has been calculated considering the standard deviation of the two parameters (*a* and *b*) of the linear fit and the general formula of error propagation using as independent variables the shift, *a* and *b*.

The mean values of the wine AS calculated with the PSOM are in excellent agreement with the same values obtained with the official method; **Figure 7** shows the accuracy of the wine AS predicted through the two alternative methods: PSOM and FTIR. The AS variation (i.e., error bars), obtained with the PSOM, can be extensively improved by increasing the number of measurements for each single wine, but the values reported in **Table 2** are already in accordance with the limits imposed by European law to the wine producers: the difference between the real AS and the one declared on the bottle label should be within 0.5%. The other analytical parameters, limit of detection (LOD) and limit of quantitation (LOQ), of the PSOM alcohol meter are not reported since they cannot be univocally calculated; indeed, several factors, such as the narrowness of the microcavity fwhm and the resolution of the spectrophotometer, can extensively influence and improve the detection capability of the alcohol meter. Notwithstanding, we can furnish an estimation of the PSOM sensitivity value, which can be below 0.2% (v/v ethyl alcohol) considering a spectral resolution of 0.04 nm and the slope of the calibration curve reported in **Figure 6**.

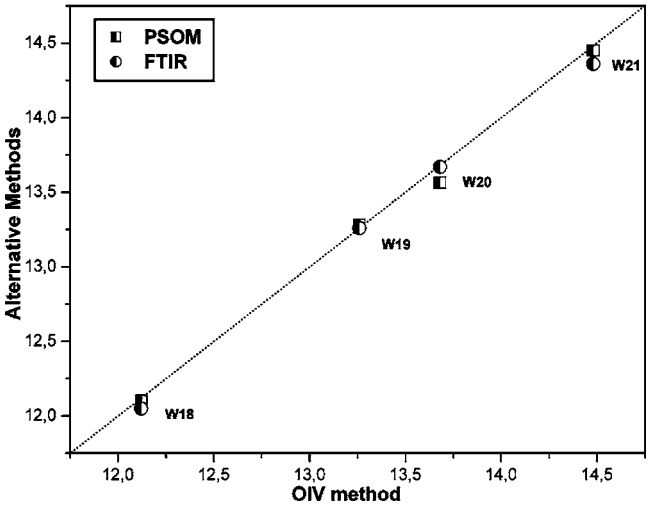

Figure 7. Accuracy of the FTIR and PSOM method: PSOM and FTIR values of AS (squares and circles, respectively) vs reference/true values of AS (OIV method).

We have developed an innovative alcohol meter based on an optical device that is able to measure the wines' AS in a short amount of time (about 15 min for one wine with five measurements), without sample pretreatments and with a high accuracy (**Figure 7**); moreover, this alcohol meter can be used by unskilled people, due to its simple working procedure, becoming an important technological upgrade for the drink industry and an extremely useful tool for wine producers performing a routine analysis.

ACKNOWLEDGMENT

We thank Stefano Borini of the Istituto Nazionale di Ricerca Metrologica for advice and fruitful discussions.

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Received for review March 19, 2007. Revised manuscript received May 24, 2007. Accepted May 28, 2007.

JF070809W