FAST ENZYMATIC METHOD FOR ACETALDEHYDE DETERMINATION IN WINE QUALITY CONTROL

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abstract: A fast enzymatic method for acetaldehyde determination applied for wine quality control is described. Analytical determination is based on the determination of variation of concentration of NADH formed in the enzymatic reaction catalysed by the aldehyde dehydrogenase in the first 20 sec. The calibration curve for acetaldehyde was linear in the 0.02 to 0.3 mM range. Determination of acetaldehyde in white wine samples was realised.

Introduction

Acetaldehyde represents one of the components in the oxidative chain of alcoholic fermentation. Acetaldehyde is formed also in the processes of wine aging by oxidation of ethanol, being the intermediary between ethanol and acetic acid.

Presence of acetaldehyde in wines is related to the wine oxidative processes. Antioxidant capacity of wines is correlated with the concentration of acetaldehyde. This is why acetaldehyde is one of the main parameters in the wine quality control. Acetaldehyde concentration is closely related with the SO_2 content of the wine. A very stable combination is formed between SO_2 and acetaldehyde ($K = 2.4 \cdot 10^{-6}$). Free SO_2 is responsible with the antioxidant activity.

The Romanian wines have an acetaldehyde concentration form 20 to 2000 mg/L for some oxidative wines. Colour, taste and wine bouquet (flavour) are affected by the content of acetaldehyde. Acetaldehyde is responsible also for alcoholic intoxication because it is a very reactive compound towards proteins, aminoacids and glutathione. For exported Romanian wines, one of the main criteria is a lowest acetaldehyde concentration.

Standard chemical methods for determination of acetaldehyde [1,2] are not expensive, relatively rapid. Chemical method has the disadvantage of a supplementary distillation step and a higher detection limit. Colorimetric methods [3-5], gas chromatography [6] or enzymatic methods [7-10] were also developed. Enzymatic methods have the advantage of selectivity and rapidity. Almost all enzymatic methods are based on use of aldehyde dehydrogenase.

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Aldehyde dehydrogenase (E.C 1.2.1.5) catalyses the oxidation of aldehydes to carboxylic acids in the presence of NAD or NADP according to reaction [11,12]:

$$\text{R-CHO} + \text{NAD(P)}^+ + \text{H}_2\text{O} \xrightarrow{\text{aldeyde dehydrogenase}} \text{R-COOH} + \text{NAD(P)H} + \text{H}^+ \quad (1)$$

Unlike the majority of enzymatic reactions, the reaction catalysed by this enzyme is practically an irreversible reaction [13] and it could be used for quantitative determination of acetaldehyde.

Acetaldehyde dehydrogenase (AlDH) can oxidise many aliphatic and aromatic aldehydes like: acetaldehyde, propionandehyde, benzaldehyde, formaldehyde, etc. in the presence of NAD or NADP. Acetaldehyde manifests the highest affinity for this enzyme ($K_M = 9.10^{-6}$ M). Potassium ions and mercaptoethanol are used to activate and maintain the activity of enzyme [14].

Assay time and cost of determination are very important from practical point of view. Enzymatic determination using steady-state method requires till 5 minute reaching the steady-state signal.

In this paper a fast spectrometric method for acetaldehyde determination was developed for control of acetaldehyde concentration in wine samples. The rate of NADH formation is determined spectrometrically at 340 nm, by calculating the variation $\Delta A/\Delta t$ which is proportional with the concentration of acetaldehyde from the sample.

Experimental

Materials

Acetaldehyde dehydrogenase (E.C 1.2.1.5) and β-NAD^+ were purchased from Sigma, and acetaldehyde from Fluka. All other reagents were of analytical grade. Standard solutions of acetaldehyde were prepared in phosphate buffer *p*H=9 with KCl 0.1 M.

White wine samples were purchased from I.C.V.V. Valea Calugareasca.

Equipments

Spectrometric measurements were performed with disposable cuvettes of 1.5 mL using a Cary 100 Bio spectrometer, with dual cell Peltier accessory for temperature and stirring control of reaction mixture (1.5 mL), data display and recording were Cary Win UV software version 3.0. Measurements were realised in the first 20 sec.

All cuvettes were previously kept for 2 minutes to reach the set-up temperature.

Results and Discussion

Optimisation of coenzyme concentration

The rate of the enzymatic reaction is influenced by the amount of coenzyme, NAD^+, which is consumed during the oxidation of acetaldehyde. Excess of NAD^+ is necessary in order to have a zero degree reaction order towards the co-substrate.

Concentrations of NAD^+ from 0.1 to 1 mM were tested and experimental results are presented in Fig. 1.

The reaction rate becomes independent from the concentration of NAD^+ starting with 0.5 mM. A concentration of 0.7 mM NAD^+ was considered a good compromise between the cost of one determination related with the consumption of this reagent and saturation of the enzyme with coenzyme and it was used for further experiments.

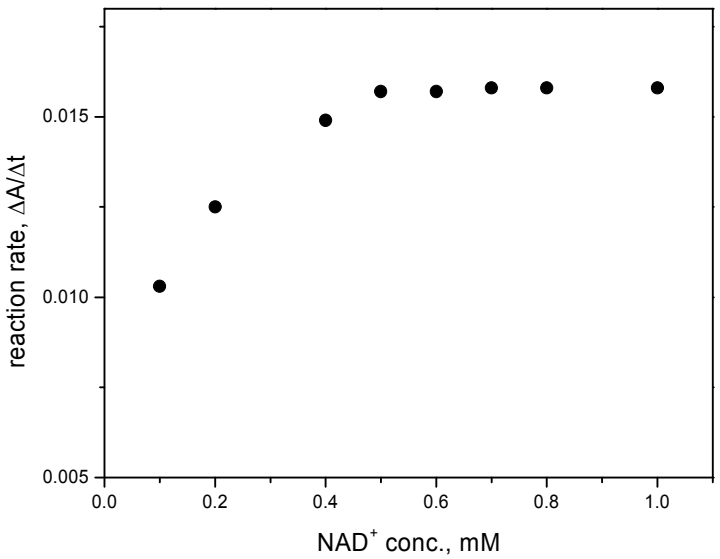

Fig. 1. *Optimisation of coenzyme concentration (temperature 25 °C, pH=9.00, acetaldehyde 0.1 mM).* Each point represents the average of 3 determinations.

Effect of temperature

It is well known that enzymes activity is affected by temperature. Depending of the source of enzyme the optimal temperature can vary. Effect of temperature upon the enzyme reaction rate was studied in the range of 20 – 35°C. Fig. 2 shows that the maximum rate is reached for a temperature of about 35°C. A minor difference was observed between reaction rate for 25 and 35°C. Therefore temperature of 25°C was used for further experiments, taking into account also the fact that at this temperature the temperature control is not a critical point.

Effect of *p*H

Optimal *p*H of the acetaldehyde dehydrogenase reported by the supplier is 9. Working *p*H is important not only from the point of view of the enzyme activity but also affect the state of the substrate. For wine samples, total amount of acetaldehyde should be determined. Concentration of free acetaldehyde is relatively low and the complex with SO_2 has to be decomposed. Alcalin *p*H discriminates in favour of complex decomposition. Therefore a *p*H=9 was used for all determinations. A *p*H=7 could be used for determination of free acetaldehyde but a calibration must be performed at this *p*H.

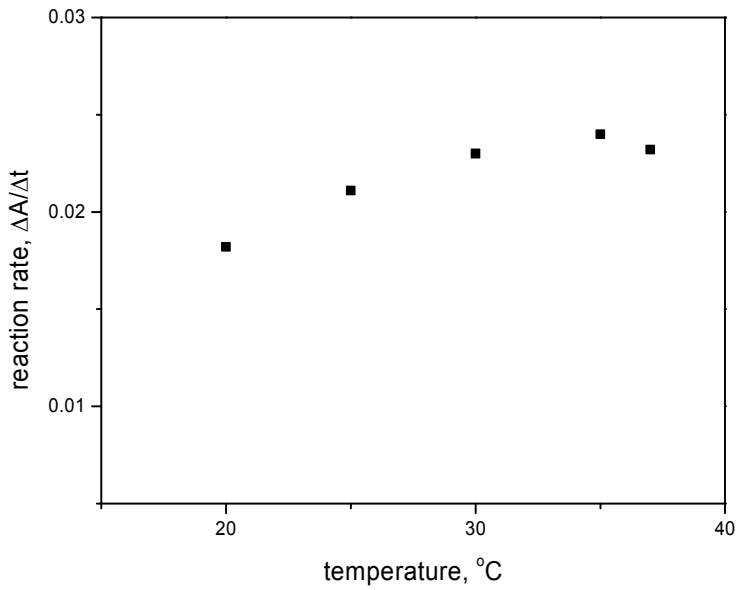

Fig. 2. *Effect of temperature on enzyme reaction rate* (*p*H=9.00, acetaldehyde 0.2 mM). Each point represents the average of 3 determinations.

Substrate inhibition

It is reported that higher concentrations of acetaldehyde produce an inhibition of the enzyme [15]. This effect was demonstrated by using different concentrations of acetaldehyde. Kinetics measurements, for concentration of acetaldehyde between 0.066 and 0.4 mM, are presented in Table 1.

Table 1. Inhibition effect of substrate (*p*H=9, t=25ºC, 0.2 UI AlDH, 0.67 mM NAD^+)

Acetaldehyde conc. (mM)	Slope (reaction rate)	Effect
0,066	0.0138	-
0,133	0.0176	-
0,166	0.0192	-
0,2	0.0211	-
0,33	0.0152	inhibition
0.4	0.0124	inhibition

Enzyme inhibition occurs for acetaldehyde concentrations higher than 0.3 mM. Calibration graph should be plotted for concentration up to 0.3 mM acetaldehyde.

Calibration curve

Fig. 3 shows the calibration curve for acetaldehyde under conditions of *p*H=9.00 and 25°C. Each point represents the average for 5 determinations.

Parameters for linear regression (y=ax+b) are:

$$a = 0.0104 \pm 0.00006$$

$$b = 0.053 \pm 0.003$$

with the correlation coefficient of 0.9983 and standard deviation SD=0.22.

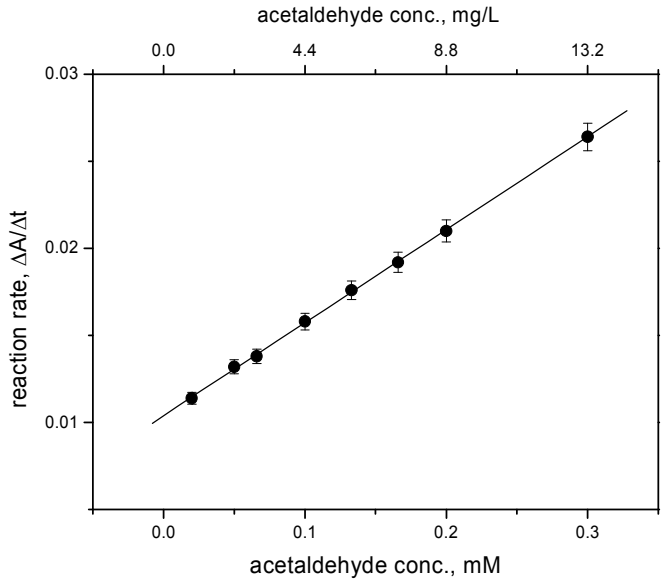

Fig. 3. *Calibration curve for acetaldehyde* (kinetic measurements, temperature 25 °C, *p*H=9.00).

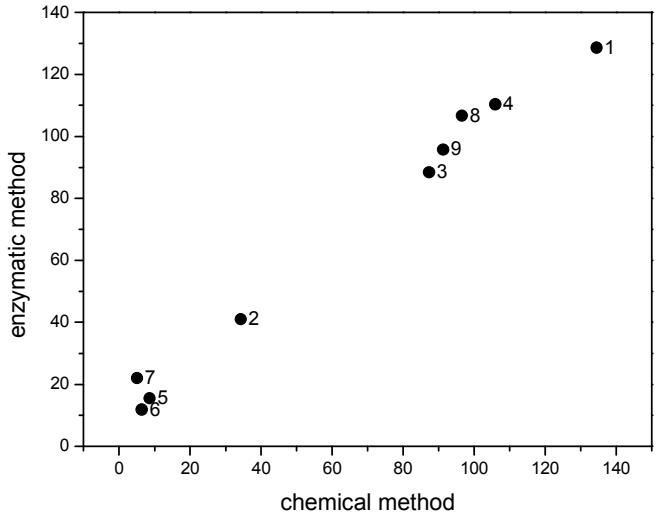

Fig. 4. *Correlation between enzymatic method and chemical method.*
5 determinations were realised for each sample. 1 - Feteasca 1997; 2 - Feteasca 1986; 3 - T.R. 1977; 4 - T.R. 1988; 5 - Chardonnay 2002; 6 - Muscat Ottonel 2002; 7 - Pinot Gris 1990; 8 - Riesling 1990; 9 - Sauvingnon 1976

Acetaldehyde determination in wine samples

Wines from I.C.V.V. Valea Calugareasca have a declared concentration of acetaldehyde between 20 and 150 mg/L. Dilutions 1/10 of the wine samples were realised before measurements.

Results for 9 wines are represented in Fig. 4 for enzymatic method and chemical method. The correlation coefficient of experimental data of 0.9948 shows a good correlation between enzymatic kinetic method and chemical method. The variation coefficient was no more than 3% for enzymatic method and 2.5% for chemical method, for determination realised in the same day.

Conclusions

A fast enzymatic method for determination of acetaldehyde from wine samples was realised. Working conditions were *p*H=9 and temperature of 25^{o}C.

Linear response was recorded in the range of 0.02 – 0.3 mM acetaldehyde. Drawback of the kinetic method is represented by inhibition effect manifested at higher substrate concentration. Method is fast and sample dilution eliminates the necessity of adjust the *p*H of the wine.

Comparable sensitivity and good correlation with chemical method were observed.

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