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# RAPID AND SPECIFIC METHOD FOR RESIDUAL OXALATES EVALUATION IN CORK STOPPERS FOR WINE BOTTLING

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## SUMMARY

An enzymic procedure is proposed for the quantification of oxalates in cork stoppers. A study of the sensibility, repeatability and accuracy of the proposed method is presented. The advantage of the proposed method over those previously recorded for oxalate quantification is its simplicity, specificity and application to a large number of samples in a minimal time (3 h). The method could be put to use by regulatory agencies, cork stopper manufacturers and the food industry to determine the residual oxalates in cork stoppers.

## INTRODUCTION

High quality wines continue to be bottled in glass botles using cork stoppers. The chemical treatment of cork stoppers involves a complex tecnology, encompassing various steps, i. e. bleaching, whitening, colouring and finishing.

When bleaching cork stoppers, several chemicals can be used (Sarti *et al.*, 1986), such as hydrogen peroxide and hypochlorites.

Oxalates are whitening products used for the precipitation of the metals remaining in the corks, resulting from the salts used in the bleaching treatment. For technical and toxicological reasons the evaluation of oxalate residues in cork stoppers is thus essential (Tanner, 1979).

Colorimetric, spectrophotometric, potentiometric, atomic absorption spectrophotometry, HPLC, gas chromatography with derivatization and enzymic methods are some quantification

procedures which have previously been applied to determination of oxalates in various biological products, such as urine, blood, serum and plants.

In an attempt to select the best method for the quantification of oxalate in cork stoppers, a comparison with the methods described in the literature was conducted. The results of this study will be published elsewhere.

As far as we know, the assay of oxalates in any biological product has proved to be very difficult and no international official method has been proposed for quantitative determination of oxalate in cork stoppers. The procedure we are describing here is an adaptation of the original enzymic method for determining urinary oxalate.

Since Portugal is a big cork producer, there is a need to develop quality control methods which will prove to be both industrially practicable as well as officially sensitive and accurate. On these terms, the selected method is believed to be the most satisfactory of the methods previously tested by us, despite its expense.

## MATERIALS AND METHODS

### *Method basis*

The basis of the method is that of the oxalate quantification in urine, which has been described by Sigma Diagnostics. The adaptation consisted of oxalate extraction from the stoppers with hydrochloric acid solution, followed by the oxalate evaluation used in the enzymic Sigma Diagnostic assay.

A hydrochloric acid extract at pH 2 of the cork stoppers is used for measuring the oxalate. The solution extracted is then shaken with an absorbent which selectively binds the oxalate. The solution is then discarded and the oxalates are eluted from the absorbent with a dilute alkali solution. The following reactions are then involved in measuring oxalate.

$$\text{Oxalate} + O_2 \xrightarrow{\text{Oxalate Oxidase}} 2CO_2 + H_2O_2$$

$$H_2O_2 + \text{MBTH} + \text{DMAB} \xrightarrow{\text{Peroxidase}} \text{Indamine Dye} + H_2O$$

The oxalates are oxidized to hydrogen peroxide and carbon dioxide by oxalate oxidase. The hydrogen peroxide reacts with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-dimethylaminobenzoic acid (DMAB) in the presence of peroxidase, yielding an indamine dye with a maximum absorbance at 590 nm.

***Chemicals***

Kit Sigma Diagnostcs for the quantitative, enzymic determination of oxalate in urine at 590 nm, Procedure N° 590 (Oxalate reagent A, containing DMAB, peroxidase and oxalate oxidase; Oxalate reagent B, containing MBTH; Oxalate extraction vial, containing absorbent; Sodium hydroxide solution, 0.2N).

Dionised water and hydrochloric acid solution pH 2.

Oxalic acid (COOH.COOH $2H_2O$) (p.a. Merck) used to prepare the standard solutions: Stock solution at 1 mg/ml and diluted standard work solutions at 10, 25, 50 and 100 $\mu$g/ml concentrations, freshly prepared from the stock solution.

***Samples***

Five representative cork stoppers from batch A (untreated), batch B, C (increased bleaching and whitening) and batch D (bleaching, whitening and finishing treatment) were used.

***Equipment***

Spectrophotometer 150-20 Hitachi.

***Extraction***

Five cork stoppers from batch A, B, C and D respectively were cut into 12 portions each and put with a steel magnet into a 250 ml Erlenmeyer flask. Hydrochloric acid solution at pH 2 was added to fill the Erlenmeyer completely to the top (about 300 ml), and it was then stoppered with a glass stopper to ensure the corks did not float at the surface but were submerged.

The extraction was carried out at 40° C, by stirring for 2 hours. After cooling at room temperature, the solution was

separated by filtration into a volumetric 500 ml flask, and the volume was made up with the washing waters. After homogenization the assay was carried out as specified in the literature of Sigma Kit.

At the same time, the porcedure was repeated with diluted standard working solutions containing 10, 25, 50, 100 $\mu$g/ml of oxalic acid.

## RESULTS AND DISCUSSION

A calibration curve was plotted from the absorption of the standards at 590 nm, versus the concentration (ranging from 2 $\mu$g/ml to 500 $\mu$g/ml. Linearity was observed between 10 $\mu$g/ml and 100 $\mu$g/ml, i. e., the work range recomended

(Linearity equation: $y = -0.005 + 0.004 \times \quad R = 1.00$)

The oxalate contents of cork stoppers, expressed as oxalic acid, were determined from the calibration curve by interpolation of the spectrophotometric absorption readings of the extraction solutions. If necessary an appropriate dilution of the extracted solution should be carried out in accordance with the sensitivity procedure mentioned below.

The study was carried out on four types of cork stoppers: natural or untreated cork (A) and three stages of bleaching (respectively B, C, D). The results obtained from the assay of the four extracted solutions are shown in Table 1. An average

TABLE 1

Results of oxalate quantification in the cork stoppers

*Resultados da avaliação dos teores em oxalatos nas rolhas de cortiça*

| Samples | N | Oxalic acid mg/cork | | Standard deviation |
|---|---|---|---|---|
| | | Average | Range | |
| A | 6 | 1.0 | 0.6-2.0 | 0.50 |
| B | 6 | 7.2 | 6.1-8.4 | 0.95 |
| C | 6 | 5.7 | 4.6-7.0 | 0.97 |
| D | 6 | 5.8 | 4.6-6.8 | 0.90 |

of 6 assays was conducted on 5 cork stoppers of each batch, and the standard deviations are included.

In a litigius society we must be sure that the results of an analysis comply with the highest qualitative and quantitative standards. We must realise that we are working on analytes which are present in complex matrices, the results of which may vary extensively due to some interferences, which cannot be ruled out by the analyst.

Interfering substances, particularly ascorbic acid (Glick, 1987; Kasidas *et al.*, 1987) homogentisic acid (Biggs *et al.*, 1986) EDTA (Thode *et al.*, 1987) and divalent metals (Laker *et al.*, 1980; Hodgkinson, 1981) were previously reported. The interferences of the former should be neglibible, but the divalent metals should be considered.

To prove the metal interferences resulting of the complexity of the cork treatment and its preservation, some assays were carried out by adding hydrogen peroxide, potassium metabisulfite and calcium hypochlorite $Ca(ClO)_2$ to the extraction solution of the cork stoppers. When 0.01 mg/ml of hydrogen peroxide or 0.01 mg/ml of calcium hypochlorite were added no effect was observed. However the addition of 0.01 mg/ml potassium metabisulfite decreased the spectrophotometric absorption readings.

A study of the sensitivity evaluation was carried out on standard solutions of oxalic acid with concentrations from 1 $\mu$g/ml to 500 $\mu$g/ml (Table 2). It was set out at 5 $\mu$g/ml.

TABLE 2

Results of the absorption standard study
*Estudo da absorvência dos padrões*

| Standards | N | Oxalic acid ($\mu$g/ml) | Espect. absorption (E) | Standard deviation |
|---|---|---|---|---|
| 1 | 2 | 2 | 0 | 0 |
| 2 | 2 | 5 | 0.008 | 0.0002 |
| 3 | 2 | 10 | 0.038 | 0.0001 |
| 4 | 2 | 25 | 0.100 | 0.0001 |
| 5 | 2 | 50 | 0.220 | 0.0002 |
| 6 | 2 | 100 | 0.400 | 0.0001 |
| 7 | 2 | 250 | 0.510 | 0.0002 |
| 8 | 2 | 500 | 1.017 | 0.0001 |

The accuracy was carried out by the addition method in cork sample $A_1$ and $C_1$ to which 10, 25, 50, 100 $\mu$g/ml of oxalic acid were added (Table 3).

TABLE 3

Results of the accuracy study by addition method
*Estudo da exactidão pelo método das adições*

| Sample | Original oxalate ($\mu$g/ml) | Added ($\mu$g/ml) | Found ($\mu$g/ml) | Recovery (%) |
|---|---|---|---|---|
| $A_1$ | 9 | 10 | 18 | 90 |
| $A_1$ | 9 | 25 | 32 | 92 |
| $A_1$ | 9 | 50 | 55 | 92 |
| $A_1$ | 9 | 100 | 105 | 95 |
| $C_1$ | 48 | 10 | 57 | 90 |
| $C_1$ | 48 | 25 | 71 | 92 |
| $C_1$ | 48 | 50 | 93 | 90 |
| $C_1$ | 48 | 100 | 144 | 96 |

The precision, in terms of repeatability, was carried out on cork samples A and B, the assay being repeated ten times in each batch (using the Nalimov test). The aberrant values (Gottschalk, 1979) were found by further calculation (Table 4).

TABLE 4

Statistical data for oxalate quantifications in cork stoppers
*Estudo estatístico referente à avaliação de oxalatos em rolhas de cortiça*

| | | Oxalic acid mg/cork | | | |
|---|---|---|---|---|---|
| Sample | N | Average | Standard deviation | Variation coef. (%) | Repeatability (%) |
| A | 8 | 1.1 | 0.09 | 8.8 | 29.73 |
| B | 10 | 7.2 | 0.64 | 8.9 | 32.30 |

As far as we know, this enzymic method has not been previously described for oxalate determination in cork stoppers and is satisfactory for the oxalate quantification in natural and treated cork stoppers.

The advantage of the proposed method over the ones previously recorded for oxalate quantification is its simplicity, specificity and application to a large number of samples in a short period of time (3 h). Thus, the method could be put to use by regulatory agencies, cork stopper manufacturers and the food industry to determine the residual oxalates in cork stoppers, especially to distinguish between natural and oxalate treated cork stoppers.

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## RESUMO

**Método rápido e específico para determinação de oxalatos em rolhas de cortiça para vinhos engarrafados**

Propõe-se um método enzimático para avaliação de oxalatos em rolhas de cortiça. Apresenta-se um estudo da sensibilidade, repetibilidade e recuperação do método. A sua vantagem sobre os métodos conhecidos é a simplicidade, especificidade e aplicação a um grande número de amostras num tempo relativamente curto (3 h). O método pode ser aplicado nas empresas produtoras de rolhas e na indústria alimentar utilizadora, para determinar o resíduo de oxalatos em rolhas de cortiça.

## RÉSUMÉ

**Rapide et especifique méthode pour l'analyse des oxalates en bouchons de liége pour vins en bouteille**

On établi une technique enzimatique pour quantifier les oxalates en bouchons de liége.

On étudie la sensibilité, la répétabilité et la récupération que le méthode offre. Ses advantages sont la simplicité, especificité et la possibilité de leur aplication sur an grand numéro d'échantillons et la rapidité d'exécution (3 h). On peut employer le méthode aux industries de bouchons et aux industries alimentaires utilizateurs, pour quantifier les oxalates.

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