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Analytical Methods

Selective methods for polyphenols and sulphur dioxide determination in wines

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ABSTRACT

A critical review to the methods recommended by international bodies and widely used in the winery industry and research studies was performed. A Laccase biosensor was applied to the selective determination of polyphenols in wines. The biosensor response was characterised and it responds mainly to o-diphenols which are the principal polyphenols responsible for the stability and sensory qualities of wines. The spectrophotometric method to determine free and total sulphur dioxide recommended for beers was applied directly to wines. A sampling of 14 red and white wines was performed and they were analysed for biosensor polyphenol index (I_{BP}) and sulphur dioxide concentration (SO_2). The antioxidant capacity by the $ABTS^{\cdot+}$ spectrophotometric method was also determined. A correlation study was performed to elucidate the influence of the polyphenols and SO_2 on the wines stability. High correlations were found between I_{BP} and antioxidant capacity and low correlation between SO_2 and antioxidant capacity. To evaluate the benefits of wine drinking a new parameter (I_{BP}/SO_2) is proposed.

1. Introduction

Wine is a complex mixture of several hundred compounds present at different concentrations. Many of them are found at very low concentrations, even though they play an important role in wine evolution and quality. Usually, the parameters to be analysed are simplified and are focused on the sample evolution, sensory characteristics or to fulfil food laws. Among the parameters more often analysed are polyphenols index (I_{BP}) and sulphur dioxide (SO_2).

Many analytical methods recognised by the international community as official methods of analysis are useful for monitoring wine composition, but in many cases obsolete and show lack of selectivity. However, there are methods described in the scientific literature to provide better analytical results, therefore is time to change, to break the inertia and to move to more advanced methodologies.

Polyphenols compounds are a complex group of substances of plant origin. The basic features of all polyphenolics are the presence of one or more hydroxylated benzene rings. The two main groups of polyphenolics are phenolic acids and flavonoids (Blasco, Rogerio, Gonzalez, & Escarpa, 2005). Their concentration in wines depends on grape variety, geographical origin, soil type, harvest and winemaking technique. These compounds are responsible of the sensory properties of the wines. Due to its antioxidants properties they have been associated with reduced risk of cancer, heart disease and diabetes (Sies, 2010).

The individual identification of all polyphenols is not possible and furthermore, it would be a difficult task because of their chemical complexity. Some authors determine individually chemical compounds (Russo, Andreu-Navarro, Aguilar-Caballos, Fernández-Romero, & Gómez-Hens, 2008) but in such a complex samples could be more useful to determine groups of similar compounds. Very often these results can be more valuable to assess the quality of wines. Therefore, it is important to underline the interest of "polyphenols indexes" (Gamella, Campuzano, Reviejo, & Pingarron, 2006). Traditionally, the term "total phenolics contents" refers to the results obtained by spectrophotometric methods, specially the Folin–Ciocalteu method. The reagent used in this method is an oxidant mixture that reacts with reducing substances without selectivity to form chromophores that can be detected spectrophotometrically. In the case of the wines, yields an overestimation of the total polyphenolic content, as it is known, the main interferents are: sulphur dioxide, reducing sugars and ascorbic acid. In spite of that, many authors to validate a method to determine polyphenolic content in food samples compare with the ones obtained with the Folin–Ciocalteu method. In all cases, as expected, the results with

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this method are higher than when a more selective method for polyphenols is used. Therefore, the Folin–Ciocalteu method is a measure of the reducing capacity rather than a determination of polyphenols (Martinez-Periñan et al., 2011). In the cases, when the relationship polyphenols versus other reducing agents is high, the positive error is lower than when this relationship is low.

In recent times, different electrochemical methods have been proposed for the characterisation of polyphenols in wine on the basis that practically all polyphenolic molecules present in wine are electrochemically active (Sanchez-Arribas, Martinez-Fernandez, & Chicharro, 2012). Cyclic voltammetry was the first electrochemical method used for characterisation of polyphenols and determination of total polyphenols content in wines (Marijan, Novak, & Jakobek, 2009). Flow injection analysis with electrochemical detection for determination of total polyphenols in wines (Blasco et al., 2005; Gamella et al., 2006) and differential pulse voltammetry (Marijan et al., 2009) has also been used. These methods also can present interferences from other compounds with similar redox potentials. Biosensors have been proposed as efficient analytical tools for the detection of polyphenol compounds, exhibiting advantages mainly a high selectivity. An amperometric Laccase biosensor with the enzyme immobilised onto a glassy carbon electrode has been described for wines (Gamella et al., 2006) and also an amperometric determination by Laccase immobilised onto silver nanoparticles/zinc oxide nanoparticles modified gold electrode (Chawla, Rawal, Kumar, & Pundir, 2012). In this work, a Laccase–Sonogel-Carbon biosensor (Lac/SNGC) previously developed by us (ElKaoutit et al., 2008) and applied to beers has been used to determine polyphenols in wines. A study of the biosensor response to the different polyphenols present in wines was performed.

The sulphur dioxide is found naturally in wines at low concentrations, besides is added to prevent oxidation and bacteria growth and to control enzymatic reactions during elaboration and storage. Without this additive is not possible to guarantee the wine quality, nevertheless, an excess can spoil wine since it produces discolouration, gives a spicy odour and change the flavour. Sulphur dioxide has been the subject of legislation as food additive since the discovery that it causes asthmatic attack, allergic reactions to hypersensitive people and dermatological problems (Vally, Misso, & Madan, 2009). In the USA and EU, a directive requires food manufactures to label if it is present at concentration higher than 10 mg L^{-1} in prepacked foods (Spricigo et al., 2009), since it is potentially toxic.

The sulphur dioxide can be present in wine under two forms: free (HSO_3^- or SO_2) or bound to carbonyl and unsaturated compounds and to phenols (Barbe, De Revel, Joyeux, Lonvaud, & Bertrand, 2000) existing in a reversible equilibrium between the two forms. The free sulphur dioxide has antiseptics and reductant properties and its levels should be adjusted before packing.

As above mentioned, it is necessary to develop a precise, sensitive and selective method for sulphur dioxide determination. Most of the used methods are based on sulphur dioxide oxidation to sulphuric acid. The International Organisation of Vine and Wine method is based on the sulphur dioxide separation by a nitrogen or air stream followed by an oxidation with hydrogen peroxide and the sulphuric acid formed is titrated with sodium hydroxide. The differentiation between free and bound sulphur dioxide is performed by temperature (10 °C for free and 100 °C for bound sulphur dioxide). The quantitative separation is not assured and depends on the sulphur dioxide wine content. Its accuracy, precision and reproducibility are not adequate.

The Ripper method (Ripper, 1892) is the most widely used in wineries; it is based on the sulphur dioxide titration with iodine and starch as indicator. It presents two drawbacks: difficulties to detect colour changes, especially in red wines and positive errors due to the interference of polyphenols, ascorbic acid and other reductants.

It is possible to find more advanced methodologies based on instrumental techniques: electroanalytical techniques (Baldo, Salvatore, & Mazzocchim, 1994; Kawamura et al., 1994), piezoelectric sensors (Palenzuela, Simonet, Rios, & Valcarcel, 2005), atomic spectroscopic techniques (Cmelik, Machát, Niedobová, Otruba, & Kanický, 2005; Huang, Becker-Ross, Florek, Heitmann, & Okruss, 2005) and chromatographic techniques (Lim et al., 2014). In the case of beers, sulphur dioxide is determined by UV–Vis molecular absorption spectrophotometry after the reaction of sulphur dioxide with organic reagents which provides a high selectivity; the most commonly used is p-rosaniline-formaldehyde (method recommended by the American Society of Brewing). This method has been applied to wines but separating previously the sulphur dioxide by pervaporation and followed by flow injection analysis (Mataix & Luque de Castro, 1998).

In this work, a method is proposed to determine directly sulphur dioxide in wines based on the reaction with p-rosaniline-formaldehyde with a high selectivity and that allows the differentiation between free and bound sulphur dioxide.

These two selective methods for polyphenols and sulphur dioxide have been applied to 14 samples of wine (reds and whites). The antioxidant capacity by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method has also been determined. The results agree with the ones found in bibliography. With the help of statistic tools, the influence of polyphenols and sulphur dioxide on stability and wine quality was studied. High correlations between antioxidant capacity and polyphenol index have been found, it indicates the potential role of the Lac/SNGC biosensor for determining polyphenols responsible of the antioxidant capacity in red wines.

2. Experimental

2.1. Reagents

2.1.1. Electrochemical transducer preparation and biosensor fabrication

Acetic acid glacial 100% (Merck, Darmstadt, Germany); sodium acetate (Merck, Darmstadt, Germany); methyltrimethoxysilane (MTMOS) (Merck, Hohenbrunn, Germany); nafion-perfluorinated ion-exchange resin 5% (w/v) in a mixture of lower aliphatic alcohols and water was obtained from Aldrich (Steinheim, Germany); glutaraldehyde 25 wt.% solution water (Aldrich, Steinheim, Germany); gallic acid monohydrated (Sigma–Aldrich, Steinheim, Germany); Laccase from trametes versicolor (Fluka, Germany); graphite powder, natural, high purity 200 mesh (Alfa Aesar, Karlsruhe, Germany).

All reagents were of analytical grade and used as received without further purification. Nanopure water was obtained by passing twice-distilled water through a Milli-Q system (18 MΩ cm, Millipore, Bedford, MA). Glass capillary tubes, i.d. 1.15 mm, were used as the bodies of the composite electrodes.

2.1.2. Polyphenol assays

Gallic acid 98% (Sigma–Aldrich, Steinheim, Germany); quercetin (Sigma–Aldrich, Steinheim, Germany); rutin hydrate 94% (Sigma–Aldrich, Steinheim, Germany); tannic acid 90% (Fluka, Buchs SG, Switzerland); ferulic acid 98% (Fluka, Buchs SG, Switzerland); (+) catechin 96% (Sigma–Aldrich, Steinheim, Germany); (−)epicatechin 90% (Sigma–Aldrich, Steinheim, Germany); tyrosol 95% (Sigma–Aldrich, Steinheim, Germany); caffeic acid 95% (Fluka, Buchs SG, Switzerland); vanillic acid (Fluka, Buchs SG, Switzerland); syringic acid (Kodac Rochester, NY); p-coumaric acid 95% (Sigma–Aldrich,