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Cyclic voltammetry: A tool to quantify 2,4,6-trichloroanisole in aqueous samples from cork planks boiling industrial process

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ABSTRACT

Chloroanisoles, namely 2,4,6-trichloroanisole, are pointed out as the primary responsible of the development of musty off-flavours in bottled wine, due to their migration from cork stoppers, which results in huge economical losses for wine industry. A prevention step is the detection of these compounds in cork planks before stoppers are produced. Mass spectrometry gas chromatography is the reference method used although it is far beyond economical possibilities of the majority of cork stoppers producers. In this work, a portable cyclic voltammetry approach was used to detect 2,4,6-trichloroanisole extracted from natural cork planks to the aqueous phase during the cork boiling industrial treatment process. Analyses were carried out under ambient conditions, in less than 15 min with a low use of solvent and without any sample pre-treatment. The proposed technique had detection (0.31 ± 0.01 ng/L) and quantification (0.95 ± 0.05 ng/L) limits lower than the human threshold detection level. For blank solutions, without 2,4,6-trichloroanisole addition, a concentration in the order of the quantification limit was estimated (1.0 ± 0.2 ng/L), which confirms the satisfactory performance of the proposed methodology. For aqueous samples from the industrial cork planks boiling procedure, intra-day repeatabilities were lower than 3%, respectively. Also, 2,4,6-trichloroanisole contents in the aqueous samples determined by this novel approach were in good agreement with those obtained by GC–MS (correlation coefficient equal to 0.98), confirming the satisfactory accuracy of the proposed methodology. So, since this novel approach is a fast, low-cost, portable and user-friendly method, it can be an alternative and helpful tool for *in-situ* industrial applications, allowing accurate detection of releasable 2,4,6-trichloroanisole in an earlier phase of cork stoppers production, which may allow implementing more effective cork treatments to reduce or avoid future 2,4,6-trichloroanisole contaminations of wine.

1. Introduction

Wine contamination with fungal aromas is a major problem for the wine industry, namely the organoleptic defect usually (and erroneously) designated as cork taint [1]. Although other sources of contamination exist [1,2] cork is pointed out as its main cause, since cork stoppers would be the source of wine contamination by chloroanisoles, specially 2,4,6-trichloroanisole (2,4,6-TCA), that confers a very unpleasant fungal aroma to the wine even at concentrations of 2–4 ng/L [3]. Different detection (1.4–4.6 ng/L) and recognition thresholds (4.2–10 ng/L) have been reported [3]. The former can be defined as the minimum value of a sensory stimulus needed to give rise to a sensation and the latter as the minimum value of a sensory stimulus permitting identification of the sensation perceived [1]. However, other chemical compounds, like 2,4,6-tribromoanisole, 2-methoxy-3,5-dimethylpyrazine, geosmine, guaiacol, 1-octen-3-one, 1-octen-3-ol or 2-methyl-isoborneol, are also able to taint the wine with fungal off-odours [4,5].

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2,4,6-TCA is a metabolite formed from the biomethylation of chlorophenol presented in contaminated environment, usually by filamentous fungi, growing on cork [6]. To prevent the contamination of bottled wine with 2,4,6-TCA, manufacturers monitor its level in cork stoppers using two approaches: quantification of 2,4,6-TCA in cork stoppers or in the water used during the boiling procedure of cork planks before cork stoppers production. The latter case, may allow increasing cork time treatments or implementing new cork treatments, and, specially, avoid the cross-contamination of cork processed by means of contaminated boiling water. In either case, solid-phase microextraction (SPME) followed by the quantification of 2,4,6-TCA using gas chromatographic (GC) analysis with mass spectrometric (MS) detection or electron capture detection (ECD) are the most common quality control methods used by cork stoppers manufactures and cellars [7]. Sample preparation step is required due to the complexity of the matrix (e.g., wine, boiling cork water, washing cork stoppers water, cork stoppers or cork planks) and the low 2,4,6-TCA concentration expected [8,9]. For example, Patil et al. [10] developed a simple, fast, efficient, precise and cheap sample preparation method, based on dispersive solid-phase extraction, for the determination of the 2,4,6-TCA residues in white and red wine, using GC–MS with a detection limit lower than 10 ng/L. Márquez-Sillero et al. [11,12] were able to quantify 2,4,6-TCA in wine samples using ionic liquid-based single-drop microextraction together with ion mobility spectrometry [11] or single-drop ionic liquid microextraction coupled with multicapillary column separation and ion mobility spectrometry detection [12], with limits of detection of 0.2 and 0.01 ng/L, respectively. More recently, Karpas et al. [13] have used ion mobility spectrometry to detect 2,4,6-TCA in wine, after preconcentration and pre-separation steps. The work carried out by Schmarr et al. [14] showed that solid-phase extraction followed by multidimensional GC–MS could be applied to detect trace levels (<1 ng/L) of corky off-flavour compounds in wine samples, namely 2,4,6-TCA, well below olfactory thresholds reported for these analytes. Other pre-concentration approaches have been proposed: pervaporation [15], pressurised liquid extraction [16], supercritical fluid extraction [17], SPME [18–24], stir bar sorptive extraction [25,26], single drop microextraction [27], dispersive liquid–liquid microextraction [28,29], ultrasound-assisted emulsification microextraction [30], microwave assisted extraction [31] and microwave assisted extraction combined with dispersive liquid–liquid microextraction [9]. Recently, other methodologies rather than GC–MS based techniques have been proposed to detect and quantify 2,4,6-TCA mostly in wine. Immunoanalytical techniques [32,33] were developed and applied allowing the detection of 2,4,6-TCA, although in ranges well above the human detection threshold for wine.

Regarding cork samples, fewer works have been published so far. Juanola et al. [34] quantified 2,4,6-TCA in cork stoppers (both spiked non-contaminated corks and naturally contaminated cork) using a GC–ECD apparatus, after solid phase microextraction. The proposed procedure allowed quantifying 2,4,6-TCA concentrations ranging from 0.08 and 105.01 μg/kg. Nevertheless, the methodology used had high variability even when quantifying 2,4,6-TCA in control and spiked cork samples. Ezquerro et al. [35] developed an analytical method based on pressurised fluid extraction and GC–MS to determine 2,4,6-TCA in three naturally-tainted cork stopper samples, obtaining relative standard deviation percentages (RSD%) between 10% and 20%. Riu et al. [36] proposed a method for quantifying chloroanisoles, including 2,4,6-TCA in cork using headspace solid-phase microextraction and GC–ECD. The method allow determining the total amount of these compounds in cork stoppers (e.g., natural, agglomerated and agglomerated with disks) with a quantification limit for 2,4,6-TCA of 8.6 μg/kg, with good recoveries (between 90% and 106%), repeatabilities ($4\% < \text{RSD} < 13\%$) and intermediate precision ($5\% < \text{RSD} < 14\%$). Vlachos et al. [37] developed an instrumental method for 2,4,6-TCA analysis in cork stoppers, based on headspace SPME and GC coupled with an ECD. Although the method showed satisfactory linearity, repeatability (RSD% equal to 5.72%) and sensitivity, with limit of detection of 0.366 ng/L, these authors identified several matrix effects causing significant bias to the quantitative analysis of 2,4,6-TCA in cork soak. Vestner et al. [31] developed a microwave assisted extraction method for the analysis of 2,4,6-TCA in cork stoppers using stable isotope dilution assay in combination with stir bar sorptive extraction followed by GC–MS detection in the soaks samples, with a detection limit of 0.5 ng L^{-1}. Prat et al. [38] proposed a tool for sensory classification of cork stoppers based on the analysis of the volatile fraction of aqueous cork macerates, including 2,4,6-TCA, of tainted and non-tainted agglomerate cork stoppers by headspace SPME-GC. Olivella et al. [39] used GC–MS to quantify 2,4,6-TCA present in preconcentrated aqueous solution of cork soaks. Schmarr et al. [14] quantified the presence of trace levels of 2,4,6-TCA in cork soak samples using solid-phase extraction followed by multidimensional GC–MS. More recently, Slabizki and Schmarr [40] used a multidimensional GC–ECD to quantify corky off-flavour compounds at ultra trace level (low ng/L).

However, all these analytical methods are usually beyond the economic and technical possibilities of most cork producers, which are typically micro and small familiar enterprises, and are only applied to analyze a few samples of the final product [41]. So, finding a fast, simple and economic portable analytical method to quantify 2,4,6-TCA in aqueous solutions collected during cork planks industrial treatment, with a minimal sample preparation, which could be applied *in-situ*, is still a challenging task.

In the literature, some sensor based systems have also been proposed to quantify 2,4,6-TCA in cork samples. Moore et al. [32] developed a biosensor based on screen printed electrodes for the quantitative detection of 2,4,6-TCA using screen printed electrodes, with a limit of detection of 29 ng/L in buffer matrices, but failed to meet real sample analysis in wine. Electrochemical displacement immunosensors were proposed by Duarte et al. [33] for 2,4,6-TCA detection in buffer samples with high detection limits (200 μg/L). More recently, Varelas et al. [41] proposed a fast (3–5 min) and low-cost cellular biosensor to monitor low 2,4,6-TCA concentrations (1–12 ng/L), which was tested for assaying 2,4,6-TCA preparations in white wine and for 2,4,6-TCA extracted from cork soaks in white wine.

In this work, and based on the satisfactory preliminary results already obtained by the research team, for Acetonitrile (ACN)/water standard solutions [42], the potential use of cyclic voltammetry (CV) without any pre-treatment step, as a prevention tool, for quantifying 2,4,6-TCA (in the range of the regulatory and human detection thresholds) present in real aqueous solutions obtained from a cork boiling industrial process, was evaluated. The performance of the CV method was assessed by comparing the results obtained with those determined by a reference GC–MS method, following the requirements of the ISO standard 20752:2007 [7].

2. Materials and methods

2.1. Reagents

All reagents were of analytical grade and used as purchased. Acetonitrile (ACN, from Labscan), with a minimum purity of 99.8%, 2,4,6-Trichloroanisole (2,4,6-TCA) and tetrabutylammonium perchlorate (TBAP) were purchased to Aldrich and Fluka, respectively, both with a minimum purity of 99%. Deionised water was obtained from a TGI pure water system. Sodium chloride, from Sigma-Aldrich, had a minimum purity of 99.8%. Deuterated 2,4,6-TCA (2,4,6-TCA-d5), was