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Eletrochemical reduction of patulin and 5-hydroxymethylfurfural in both neutral and acid non-aqueous media. Their electroanalytical determination in apple juices

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ABSTRACT

The electro-reduction of patulin mycotoxin and 5-hydroxymethylfurfural at glassy carbon electrodes in acetonitrile +0.1 mol L^{-1} tetrabutylammonium perchlorate, in both the absence and the presence of different aliquots of trifluoroacetic acid is reported. 5-hydroxymethylfurfural is the most common interference in the determination of patulin in products derived from apples. The electrochemical techniques were cyclic and square wave voltammetries, and controlled potential bulk electrolysis. The number of electrons exchanged in the patulin electro-reduction of $n=1$ could be inferred from controlled potential bulk electrolysis measurements. Ultraviolet-visible and infrared spectroscopies were used to identify patulin electro-reduction product/s. A value of $(2.1 \pm 0.1) \times 10^{-5}$ cm^2 s^{-1} for the patulin diffusion coefficient was calculated from convoluted cyclic voltammograms. A method based on square wave voltammetry was developed for the quantitative determination of patulin in both fresh, and commercial apple juices in the presence of 5-hydroxymethylfurfural. Calibration curves obtained from solutions of the commercial reagent, and commercial apple juices were linear in the range from 3.0×10^{-7} to 2.2×10^{-5} mol L^{-1}. The lowest concentration measured experimentally for a signal to noise ratio of 3:1 was 3×10^{-7} mol L^{-1} (45 ppb) and a recovery percent of 84% was determined for commercial apple juices. This electroanalytical methodology appears as a good screening method for the determination of patulin in apple juices.

1. Introduction

Patulin (4-hydroxy-4H-furo [3,2–c]pyran-2(6H)-one, PAT) is a mycotoxin produced by fungi belonging to several genera, including *Penicillium*, *Aspergillus*, and *Byssochlamys* [1]. PAT is an α, β-unsaturated-γ-lactone (Scheme 1). PAT has two conjugated double bonds, and a very reactive hemiacetal group, which racemizes quickly in aqueous media, precluding isolation of the (+) and (−) optical isomers [2].

The major sources of PAT contamination are apples with blue rot, cider, and juice pressed from moldy fruit. *Penicillium expansum* is considered the main producer of PAT in apple products [3]. However, PAT is sometimes found in other fruits such as pears, apricots, peaches, and grapes, being produced in the rotten parts of these fruits [4].

PAT has a low molecular weight and is soluble in water and polar organic solvents. It is not destroyed by heat and it is stable in acid medium, but unstable in an alkaline medium or by fermentation [1,5].

During the forties PAT was isolated as a broad-spectrum antifungal antibiotic. However, subsequent studies suggested that PAT not only was toxic to fungi and bacteria, but was also highly toxic to plant and animal cells. PAT can react with terminal sulfhydryl groups of proteins and polypeptides present in food [6]. PAT has mutagenic, genotoxic, immunotoxic, and neurotoxic effects on rodents [7] and teratogenic effects on chickens [8]. Exposure of humans to PAT via consumption of infected products may result in severe toxicosis, including mutagenic, teratogenic, hepatoxic, nephrotoxic, neurotoxic, and genotoxic effects. Its acute effects include nausea, vomiting, and other gastrointestinal traumas that accompany kidney damage [7,9,10]. At high doses, PAT exhibits immunosuppressive properties [11].

The International Agency for Research on Cancer (IARC) classifies PAT in the Group 3 (an agent not classifiable as carcinogenic to humans) [12].

The Food and Drug Administration of United States (FDA) has recommended an allowable maximum level of 50 ppb for fruit juices and their products [13], due to evidence of adverse effects of PAT. To date, no epidemiological or toxicological data have been published to indicate whether the consumption of PAT is

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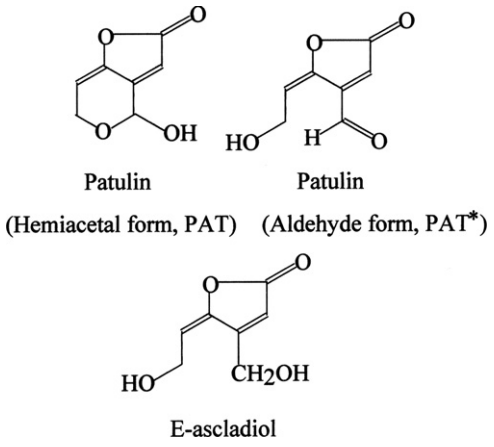

Scheme 1. Chemical structures of both patulin hemiacetal and aldehyde forms, and E-ascladiol.

dangerous to humans. However, there is an allowable level of PAT in apple products, considering that infants and young children are the major consumers of these foods, and effects of exposure to PAT for a long time are not still known [14,15].

The official method for determination of PAT is high performance liquid chromatography (HPLC) with ultraviolet (UV) detection, as is described by the Association of Official Analytical Chemistry (AOAC), item 995.10 [16,17]. The HPLC/UV or with a diode array detector (HPLC/DAD) [18] is also routinely used for quantitative determination of PAT in apple products. However, methods to confirm the presence of PAT usually include more specific detection techniques such as mass spectrometry (MS) after liquid chromatography (LC), gas chromatography (GC) separations [17,19], and high resolution gas chromatography/ high resolution mass spectrometry (HRGC/HRMS) [20]. Capillary electrophoresis is another technique to perform PAT determinations [21]. Analytical methods based on immunochemical technology are currently being investigated, but the low molecular weight of PAT presents a challenge for this area of research and development [10]. Therefore, the standard methodology to quantify PAT requires specific instrumentation and trained operators [10].

As far as we know, only one report related to the electrochemical reduction of PAT is found in the literature, with very little information [22]. On the other hand, Tanabe and Suzuki [23] reduced chemically the hemiacetal group of PAT to form E-ascladiol (Scheme 1), a compound reported to be 25% as toxic as PAT, although it has the same unsaturated bonds and γ lactone structure. In addition, it is well known that the PAT biosynthesis involves a series of condensation stages and redox reactions, several of which are catalyzed by enzymes. The E-ascladiol is involved in the last stage of PAT biosynthesis [11]. The E-ascladiol is either oxidized to PAT or non-enzymatically converted to its isomer Z-ascladiol.

On the other hand, the 5-hydroxymethylfurfural (HMF) is the most common interference in the determination of PAT in apple juices and derived products. Moreover, HMF is present at levels two or three times higher than PAT, which can cause a serious problem for the determination of PAT in natural samples [24].

In this study, we report results about the electro-reduction of PAT and HMF at glassy carbon (GC) electrodes in acetonitrile (ACN)+0.1 mol L^{-1} tetrabutylammonium perchlorate (TBAP), in the absence and in the presence of different aliquots of trifluoroacetic acid (TFA). The electrochemical techniques used were cyclic (CV) and square wave (SWV) voltammetries, and controlled potential bulk electrolysis. ACN+0.1 mol L^{-1} $NaClO_4$ was used as the reaction medium for performing measurements of controlled potential electrolysis. UV–vis and IR spectroscopies were used to identify PAT electro-reduction product/s after performing controlled potential electrolysis. A method using SWV was developed for the quantitative determination of PAT in both fresh and commercial apple juices.

2. Experimental

2.1. Reagents

PAT, HMF and TFA were obtained from Sigma Chemical Company and used as received. ACN (Sintorgan, HPLC grade), was dried over molecular sieves of 3 Å for 48 h prior to use, and then used without further purification. TBAP was obtained from Fluka (electrochemical grade). It was dried under vacuum at 60 °C, and stored in desiccators. $NaClO_4$ (Merck p.a.) was recrystallized three times in water and dried under vacuum at 180 °C, and then stored in desiccators.

Stock solutions of PAT and HMF were prepared in ACN. They were stored at 8 °C in the dark. Working solutions were prepared daily by adding aliquots of stock solution to ACN+0.1 mol L^{-1} TBAP, in the absence and in the presence of different aliquots of TFA. PAT bulk concentration (c^*_{PAT}), and HMF bulk concentration (c^*_{HMF}) were varied from 3×10^{-7} to 1.4×10^{-3} mol L^{-1}, and from 3.4×10^{-4} to 2.3×10^{-3} mol L^{-1}, respectively.

Fresh apple juices were obtained from apples purchased at a local grocery store, choosing those blocks that had no signs of putrefaction by fungi. Juices were filtered using a glass fiber paper and were called "cloudy apple juices." We also analyzed commercial apple juices, called "clear apple juices." The difference between both juices is that the "clear apple juice" is subjected to a process of depectinization, clarification, and post-filtering; while these processes were not applied to the "cloudy apple juice". PAT extraction procedure was performed in triplicate following the AOAC official method [11,16,17,24]. The reproducibility relative standard deviations (RSDR) were of 20% and 14% for "cloudy apple juices" and "clear apple juice", respectively. Value for "clear apple juice" is close to those reported by Brause et al. for similar matrixes by HPLC [16]. Three extractions were performed using 5 mL of fresh and commercial apple juices with 10 mL of ethyl acetate. The three organic phases were collected in a separator funnel and purified with 2 mL of a 1.5% Na_2CO_3 solution. As it has been reported, the rate of decomposition of patulin depends on pH i. e. values for half-life ranged from 64 h at pH 8.0 to 1310 h at pH 6.0 [25]. Thus, the recommendation suggested by the International Standard ISO 8128-1 U.S.: 1993 [26] was considered, i.e. after combining the phases, the extraction was performed with Na_2CO_3 solution as quickly as possible, i.e. 1 min to 2 min. Then, a new extraction was performed with 5 mL of ethyl acetate. The ethyl acetate extract (35 mL) was dried using 1 g of anhydrous sodium sulfate. The solution was filtered through glass fiber paper and rinsed with 5 mL of ethyl acetate. Finally, the solvent was evaporated and the extract was re-dissolved in 5 mL of ACN+0.1 mol L^{-1} TBAP for analysis. Two procedures were used in the treatment of extracts: one was conducted on the juice as obtained and, the other; the juice was spiked with a known amount of PAT, i.e., 1.29×10^{-5} mol L^{-1}. We use the standard additions method to calculate the recovery percent.

2.2. Apparatus and experimental measurements

A conventional two-compartment glass cell was used for the voltammetric measurements. The working electrode was a GC disk of 3 mm dia. (Bioanalytical Systems). Before each measurement, it was polished successively with wet alumina powder (0.3 and 0.05 μm from Fischer), rinsed copiously with distilled water, sonicated in a