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Application of voltammetric e-tongue for the detection of ammonia and putrescine in beef products

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ABSTRACT

This work describes the sensing properties of voltammetric sensors based on modified screen-printed electrodes with bisphthalocyanine and polypyrrole, respectively. The electrochemical responses of sensors to aminic compounds, including ammonia and putrescine, were analyzed. The voltammetric signals are related to the redox properties of electroactive compounds used as modifiers, strongly influenced by amine compounds from the electrolyte solution. The possibility of detecting and quantifying the amine compounds in beef extract powder was studied. Sensors are very sensitive toward amine compounds, the lowest detection limits being 1.85 μM for ammonia and 0.34 μM for putrescine. A sensors array was developed using the two types of electrodes and was subsequently used in beef freshness monitoring. As demonstrated by means of Principal Component Analysis and Partial Least Squares—Discriminant Analysis, the multisensor system is able to discriminate and classify the samples according to their storage time.

1. Introduction

Biogenic amines are formed in foods with high protein contents because of the hydrolysis processes followed by amino-acids decarboxylation [1]. The formation of the biogenic amines is an unwanted process favored by the action of microorganisms on foods with high protein content at moderate temperature [2]. On the other hand, in the technological processing of beef for extracting various dehydrated products, as is the case with beef extract powder, important quantities of biogenic amines are formed, especially putrescine and ammonia [3,4].

In physical-chemical conditions characteristic to these processes, part of ammonia volatizes, the products emanating a foul specific smell. The rest of ammonia is found dissolved, partially ionized, and has an important role in the beef products pH and toxicity [5,6].

Putrescine is formed in the process of decarboxylation of the amino acids arginine and ornithine. Putrescine is a toxic diamine, a foul-smelling organic chemical compound [7,8]. It is responsible for the specific unpleasant smell of putrefying beef [9]. The use of beef extract powder for the fabrication of instant products or culture media for various microorganisms is conditioned by the presence of biogenic amines, especially of putrescine and ammonia [10]. Therefore, the detection and quantification of putrescine and ammonia is highly important for beef dehydrated products. Also, monitoring the beef freshness is of major importance in quality control.

There are numerous means of detecting and quantifying putrescine and ammonia in food and microbiological samples. The main methods are chromatographic [11,12], spectrophotometric [13], fluorimetric [14] and chemiluminometric [15]. All the classical methods have a number of downsides related to high costs of analysis and equipment, complex pretreatment of assays, highly qualified personnel, etc.

In this context, various electrochemical methods based on chemical sensors and biosensors have been developed in view of detecting ammonia and putrescine [16–22]. The main types of electrochemical sensors used in detecting putrescine and ammonia presented in the literature, as well as their limits of detection, are included in Table 1 [16–21]. The advantages of the electrochemical methods are their simplicity, rapidity, and low costs. Also, electrochemical methods may be used as fast screening methods or for on-line, in-line or in situ monitoring of the concentration of ammonia and putrescine [23]. Nevertheless, for the analysis of complex samples, sensors array should be used, together with methods of analysis of multivariate data [24]. This type of systems named e-

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Table 1
Principal electrochemical sensors employed in ammonia and putrescine detection.

Sensitive material	Detection principle	Limit of detection	Reference
$Ru(bpy)_3^{2+}$-encapsulated silica nanoparticles on screen printed carbon surface	Electrochemiluminescence	90 nM (putrescine)	[16]
Five- or six-membered ring complexes with cupric ions	Amperometry	0.11 μM (putrescine)	[17]
Chemosensor dye—xantan derivatives with trifluoroacetophenone	Spectrophotometry	–	[18]
Polypyrrole nanowires	Cyclic voltammetry	4.78 μM (ammonia in solution)	[19]
Colorimetric sensor	Colorimetry	0.04 mg/L (ammonia in solution)	[20]
Carbon paste electrode	Cyclic voltammetry	3×10^{-4} M (ammonia in solution)	[21]

tongues have been used for the discrimination and quantification of various types of active compounds or for the study of quality or monitoring the freshness or the technological process of various food types [21,25–30].

This study has developed, characterized and used voltammetric sensors based on carbon modified by bisphthalocyanines and polypyrrole doped with various dopants for the detection and quantification of putrescine and ammonia. In optimal experimental conditions, sensors array have been used for the detection and quantification of putrescine and ammonia in beef extract powder. Another study dealt with monitoring the freshness of beef preserved in refrigeration conditions.

2. Material and methods

2.1. Chemicals and solutions

Putrescine(1,4-butanediamine dihydrochloride, ≥98%), Beef extract powder, ammonium hydroxide solution (28% NH_3 in H_2O), potassium chloride (≥99.0%), Gadolinium(III) acetate hydrate (99.9%), Dysprosium(III) acetate hydrate (99.9%), 1,2-Dicyanobenzene (Phthalonitrile 98%), pyrrole (98%), Potassium hexacyanoferrate(II) trihydrate (98.5%), Sodium nitroprusside dihydrate (98.5%), Sodium molybdate (≥98%) has been purchased from Sigma-Aldrich.

All solutions required in the experiments have been prepared with ultrapure water (18.3 MΩ × cm, Milli-Q Simplicity® Water Purification System from Millipore Corporation).

Gadolinium(III) bisphthalocyanine ($GdPc_2$) and Dysprosium(III) bisphthalocyanine ($DyPc_2$) have been synthetized and purified following previously published procedures [31]. The elemental analysis confirmed the high purity of the compound (>97% purity).

2.2. Equipments

Electrochemical measurements have been performed on a Biologic SP 150 potentiostat/galvanostat (Bio-Logic Science Instruments SAS, France). EC-Lab Express software was used for potentiostat/galvanostat control and data acquisition. A three electrode cell of 10 mL capacity with a modified carbon screen-printed electrodes (CSPE) as the working electrode, an Ag electrode as the pseudo-reference electrode, and carbon as the auxiliary electrode has been employed in the analysis of samples. Scanning electron microscopy (SEM) images have been obtained using a scanning electron microscope of Quanta 200 FEI type, with energy dispersive X-ray microanalysis (EDX) integrated system. An Elmasonic S10H ultrasonic bath has been used for dissolving and homogenization of solutions. A centrifuge Cencom II has been used in real sample pre-treatment.

2.3. Sensors set-up

Screen-printed carbon electrodes (4 mm diameter, S = 12.56 mm^2) purchased from Dropsens (www.dropsens.com, model SP 110) have been used for polypyrrole deposition or modification with bisphthalocyanine ($LnPc_2$).

Polypyrrole (Ppy) deposition has been carried out from a solution containing 0.1 M pyrrole and 0.1 M doping agent by chronoamperometry and cyclic voltammetry. A three-electrode configuration has been used. C has been the working electrode, Ag|AgCl/KCl 3 M has been employed as reference electrode and a Pt plate (1 cm^2) has been the counter electrode. Polypyrrole modified electrodes were stored into a refrigerator at 4 °C. List of doping agents and the electrochemical synthesis parameters are included in Table 2.

Bisphthalocyanines ($LnPc_2$) have been dissolved in chloroform and the concentration was 10^{-5} M. Ten microliters of the solution were cast on the surface of CSPE. After solvent evaporation, the bisphthalocyanine-modified screen printed electrodes have been stored into a refrigerator at 4 °C.

After modification, modified SPE (Ppy-CSPE and $LnPc_2$-CSPE) has been used for ammonia and putrescine detection. In all these experiments the reference and the counter electrode integrated in the commercial sensor device have been used (counter electrode – carbon, reference electrode – silver).

Cyclic voltammograms have been registered from −1.1 to +0.5 V (in the case of Ppy-CSPE) and −1.1 to +1.3 V (in the case of $LnPc_2$-CSPE), the scan started at 0 V and the scan rate has been 0.05V × s^{-1}.

2.4. Real samples

Beef extract solutions (5%) were analyzed in order to detect ammonia and putrescine. Very fresh beef meat samples have been purchased from a local traditional market. The samples have been analyzed immediately after purchasing and daily (after 24 h) during 10 days. Prior to register the voltammetric responses meat samples have been cut in small slices and blended till a paste was obtained. 4 g of meat paste has been mixed with 16 mL of 0.1 M KCl solution. The extraction of liquid phase has been carried out using filter paper followed by centrifugation at 4000 rot/min for three minutes. The supernatant has been separated and used as sample in electrochemical measurements [32].

2.5. Data analysis

The analysis of the voltammetric data recorded with the sensors array for monitoring the beef freshness has been pursued along more stages. Cyclical voltammograms in two potential domains have been recorded with both types of sensors. For combining