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A simple and rapid method for histamine determination in fermented sausages by mediated chronopotentiometry

Zorica S. Stojanović*, Jaroslava V. Švarc-Gajić

Department for Applied and Engineering Chemistry, Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

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ABSTRACT

In order to provide the basis for the development of an inexpensive, sensitive, and reliable method for histamine determination in foods, the electrochemical behavior of histamine at planar gold disc electrode was investigated by chronopotentiometry. In solutions with sufficient chloride content dual oxidative mechanism of histamine was observed and included catalytic effect of electrogenerated chlorine. Well defined signal observed at +1.15 V in 0.0025 mol/l hydrochloric acid demonstrated sensitive changes with histamine content and was attributed to histamine. Electrogenerated chlorine facilitated charge transfer between histamine and the gold electrode resulting in enhancement of sensitivity. The effects of the most important experimental parameters of the chlorine-mediated chronopotentiometric analysis, such as the type and concentration of supporting electrolyte, initial potential and oxidation current were optimised. Under optimal experimental conditions, linear response of histamine was observed in the range 2–100 mg/l with achieved limit of detection for 0.27 mg/l of histamine. Developed method was applied for histamine determination in fermented sausages.

1. Introduction

Histamine is a heterocyclic primary amine which is important mediator of numerous biological processes such as inflammation, gastric acid secretion and neural modulation. The compound is naturally present in many vegetables and fruits in amounts below toxic levels. Increased histamine levels have been observed in proteinaceous and fermented foods, such as fish, cheeses, fermented sausages, sauerkraut, wine and beer (Maintz & Novak, 2007). In food, histamine is mainly formed by microbial decarboxylation of free amino acid histidine, either by reaction catalysed by endogenous decarboxylase or as a result of uncontrolled microbial activity (Fernandes, Judas, Oliveira, Ferreira, & Ferreira, 2001). The presence of histamine in food represents a public health concern due to its physiological and toxicological effects (Švarc-Gajić, 2009). Microbial spoilage of food is accompanied with the increase in proteolytic and amino acid decarboxylase activity, thus the presence of histamine might serve as a useful chemical indicator of food spoilage (Ekici, Şekeroğlu, Sancak, & Noyan, 2004). Food intoxications resulting from ingestion of foods that contain unusually high levels of histamine have been observed and are known as histamine poisoning (Lehane & Olley, 2000). The most commonly encountered symptoms in histamine poisoning are hives, tingling, burning sensations around the mouth, gastrointestinal complaints and itching (Hungerford, 2010; Lehane & Olley, 2000). Severity of symptoms can vary considerably depending on the amount of ingested histamine and individual's sensitivity (Tsai et al., 2005).

High amounts of proteins present in fermented sausage products and proteolytic activity during ripening are a good base for decarboxylase activity of starter cultures and wild microflora. In fact, these products support the production of histamine (Bover-Cid, Schoppen, Izquierdo-Pulido, & Vidal-Carou, 1999; Gençcelep et al., 2008; Suzzi & Gardini, 2003). Histamine concentration in fermented sausages varied widely and usually ranged from 0 mg/kg to 350 mg/kg (Suzzi & Gardini, 2003). Despite the fact that fermented sausages may contain exceedingly high levels of histamine (>450 mg/kg) (Erkmen & Bozkurt, 2004), there is no legalisation dealing with limits of histamine content in these products. However, some countries have established recommendations of histamine in meat products, and levels of 100–200 mg/kg seem to be acceptable (Karovičová & Kohajdová, 2005). Since fermented sausages are worldwide popular meat products, monitoring of histamine content is very important as a quality control step and to avoid histamine-induced food intolerance.

The common methods used for determination of histamine are typically based on separation techniques such as chromatography (HPLC, TLC, GC) and capillary electrophoresis (Hwang, Wang, &

* Corresponding author. Tel.: + 381 21 485 3662; fax: +381 21 450 413.
E-mail address: zokastojanovic@gmail.com (Z.S. Stojanović).

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Choong, 2003; Jensen & Marley, 1995; Önal, 2007; Zhang & Sun, 2004; Pastore et al., 2005; Antoine et al., 2002), which are quite complex, require long analysis times and expensive instrumentation. Due to low cost and maintenance simplicity, electroanalytical techniques offer an expeditious alternative for food quality control in some specific applications. Enzyme-based electrodes with immobilised amine oxidases (Niculescu et al., 2000) and dehydrogenases (Bao, Sun, Tachikawa, & Davison, 2002; Zeng, Tachikawa, Zhu, & Davidson, 2000) were reported for histamine determination. Not many papers dealing with direct electrochemical oxidation of histamine on conventional bare electrodes such as platinum, gold or glassy carbon have been published due to high oxidation potential of histamine. Some of applied electrochemical methods for histamine quantification included derivatization of histamine with o-phthalaldehyde and 2-mercaptoethanol which yielded complex product that was subsequently electrochemically oxidised on a glassy carbon electrode at moderate potentials (Harsing, Nagashima, Duncalf, Vizi, & Goldiner, 1986). Direct cyclic voltammetric and flow injection analysis using boron-doped diamond and glassy carbon electrodes were applied for the purposes of the characterisation of the histamine electrochemical behaviour but not for its quantitative analysis (Sarada, Rao, Tryk, & Fujishima, 2000). The responses of histamine in alkaline solutions at copper- (Casella, Gatta, & Desimoni, 2001) and gold-based electrodes (Carralero, González-Cortés, Yáñez-Sedeño, & Pingarrón, 2005; Draisci et al., 1998; Draisci, Cavalli, Lucentini, & Stacchini, 1993) involving the formation of surface oxides have been utilized in detection of the histamine and other biogenic amines after their chromatographic separation. Different potential-time waveforms have been employed in electrochemical detection mode, including pulsed amperometric detection (Carralero et al., 2005; Hoekstra & Johnson, 1998), integrated pulsed amperometric detection (IPAD) (Pastore et al., 2005; Draisci et al., 1998; Draisci et al., 1993), integrated square-wave detection (ISWD) and integrated voltammetric detection (IVD) (Hoekstra & Johnson, 1998).

To our knowledge, only two previously reported studies have focused on the possibility of using chronopotentiometry for histamine determination in foods (Švarc-Gajić & Stojanović, 2010, 2011). In those studies thin film mercury electrode and thin film nickel electrode were used for electrooxidation of histamine. Although bare gold and gold-based electrodes have been widely applied in alkaline media as a part of electrochemical detection of chromatographically separated compounds for electrooxidation of histamine and other biogenic amines (Carralero et al., 2005; Draisci et al., 1998; Draisci et al., 1993), no study was reported regarding chronopotentiometric methodology of histamine determination on gold electrodes.

In this paper, a simple, sensitive and accurate method involving chlorine-mediated chronopotentiometric determination of histamine at solid gold electrode is described. The presumed mechanism of histamine oxidation at gold electrode involved electrogeneration of chlorine and subsequent histamine oxidation at electrode vicinity. The role of chlorine was to mediate the electron transfer between histamine and the gold electrode. Developed method was applied for the determination of histamine in fermented sausages. Prior method application in the analysis of real samples, the procedure of sample preparation was developed and included ultrasonically-assisted extraction and preparative column chromatography.

2. Materials and methods

2.1. Chemicals and reagents

All chemical used were analytical reagent grade (Merck, Darmstadt, Germany, pro analysis). Organic solvents used for preparative chromatography were provided by Zorka (Serbia) and silica gel (Kieselgel 60, 0.063–0.2 mesh) was from LGC Promochem (Wesel, Germany). Selected amino acids (histidine, proline, tryptophan, and tyrosine) were purchased from Merck (Darmstadt, Germany). Cadaverine, tyramine and tryptamine were obtained from Sigma (St. Louis, MO, USA). The stock standards of amino acids and amines were prepared daily in doubly distilled water, at a concentration of 0.5 g/l. Low-concentrated working solutions of amino acids and selected biogenic amines were prepared by dilution of corresponding stock solutions with supporting electrolyte. The histamine dihydrochloride standard was purchased from Fluka (Buchs, Switzerland). Stock solutions of histamine were prepared weakly by dissolving appropriate weight of histamine hydrochloride in doubly distilled water to obtain a concentration of 2 g/l of histamine free base. The histamine stock solution was stored in dark at 4 °C. Working standard solutions of histamine were prepared by appropriate dilutions of the stock solutions in the supporting electrolyte just before use. All solutions were prepared by using doubly distilled water. Laboratory accessories used were washed with nitric acid: water mixture (1:1, v/v), distilled and doubly distilled water. For histamine determination by certificated enzyme-linked immunosorbent assay (ELISA) method, the Veratox histamine kits were used (Neogen, Lansing, USA). The aliquots of prepared samples were diluted appropriately for testing with the provided kit according to the manufacturer's manual.

2.2. Instrumentation

A domestic blender (Type MKM 6000, Bosch, Munich, Germany) was used for the grinding of samples. An ultrasonic bath (Iskra, Slovenia) with working frequency of 30 kHz and power of 500 W was used in the extraction procedure. A rotary vacuum evaporator (Rotavapor-R, Büchi, Switzerland) was used to evaporate the solvent after purification. Chronopotentiometric measurements were carried out using an automatic stripping analyser (M1 analyser) of our own construction. The oxidation time (quantitative characteristic) and the oxidation potential (qualitative characteristic) were determined automatically by M1 analyser. The instrument has a program for automatic calibration of the current and voltage, with the parameter setting accuracies $\Delta E < 2$ mV and $\Delta i < 0.2$ μA. Accuracy of time measurement in oxidation time determination is 50 ms, and in all other cases 0.25 ms. Quantitative characteristic of the analyte i.e. the transition time, was measured as a time between two inflection points. Inflection points are determined by program derivation and indicated at the chronopotentiogram as horizontal dotted lines (Švarc-Gajić & Stojanović, 2010). During analytical step, the potential is measured with the frequency of 40 Hz between two inflection points. Derivative curve is internally registered, even though only standard potential vs. time curve is displayed to user. The M1 analyser was connected to Epson LX-850 printer (Fig. 1).

All electrochemical measurements were performed in a standard glass cell with a homemade gold planar disc electrode (total surface area of 7.07 mm^2) as a working electrode, a platinum wire ($Ø = 0.7$ mm, $l = 7$ mm) embedded into the glass holder as an auxiliary electrode and an Ag/AgCl electrode (3.5 mol/l KCl) as a reference electrode. All potentials quoted are relative to 3.5 mol/l Ag/AgCl reference electrode. Planar gold disc electrode was fabricated by encapsulating a gold cylinder ($d = 3$ mm, $H = 5$ mm) in a Teflon non-conducting sheath (outer diameter 8 mm). Electrical connection was established by "spot welding" a silver wire on the gold surface. Silver wire was further connected with the central line (copper wire) of coaxial cable (Fig. 1).