Studies towards the development of a novel electrochemical sensor array for the analysis of vitamins in meat

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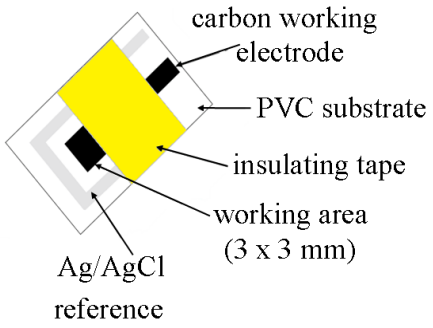
Abstract – Screen-printed carbon electrodes (SPCEs) have been investigated as disposable sensors for the simultaneous electrochemical measurement of two water-soluble vitamins; riboflavin commonly known as vitamin B_2 and pyridoxine commonly known as vitamin B_6. Reported concentrations for the vitamins in porcine muscle tissue were found to range between 0.8-4.7 mg/kg for B_2 and 1.7-5.2 mg/kg for B_6. Calibration studies using differential pulse voltammetry found that the peak response corresponded linearly over the concentration range 0.5-300 µg/mL for vitamin B_2 and 1.0-30 µg/mL for vitamin B_6. The method precision given by the coefficient of variation was 6.9 % for vitamin B_2 and 3.9 % for vitamin B_6 (*n*=3).

Key Words – Pyridoxine, Riboflavin, Screen-Printed, Voltammetry.

I. INTRODUCTION

Simple, low cost, reliable methods for vitamin analysis are required in the food and pharmaceutical industries. One promising approach is to employ disposable screen printed carbon electrodes (SPCEs) which can be mass produced in a wide range of geometries at low cost; consequently they can be considered disposable. The small sensor, shown in figure 1, can be suitable for the direct measurement of analytes. The application of SPCEs to the determination of citric acid in lime fruit has been demonstrated by Honeychurch and co-workers [1]. Many vitamins have been reported to possess electroactive behaviour in media of a specific pH. These reports have involved various electrode materials such as diamond [2], mercury [3], and glassy carbon [4]. Only a few reports have appeared which describe the application of unmodified SPCEs to vitamin analysis.

Figure 1. Electrode structure.



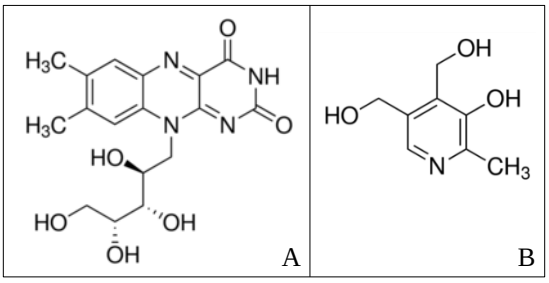
Vitamins are essential dietary components which can be found in many foods including meat products. Table 1 displays the reported concentrations for both vitamin B_2 (riboflavin) and B_6 (pyridoxine) found in porcine muscle tissue. Both vitamins are water-soluble and their structures are shown in figures 2A and 2B respectively. These compounds are often quantified in meat products using liquid chromatography [5] which requires laborious sample preparation.

The studies described here will outline the electrochemical behaviour of these two water-soluble vitamins at SPCEs with the aim to quantify them in meat produce.

Table 1. Reported concentration of Vitamins B_2 & B_6 in porcine muscle tissue (mg/kg)

Vitamin B_2	Vitamin B_6	Reference
2.3	5.2	[5]
1.4	-	[6]
1.3	-	[7]
0.83	1.73	[8]
0.9	5.1	[9]
1.3 - 4.7	-	[10]
1.22	-	[11]
1.43	3.4	[12]

Figure 2. Chemical structures of B vitamins: A) Riboflavin and B) Pyridoxine



II. MATERIALS AND METHODS

Instrumental

All voltammetric measurements were carried out with a μAutolab III potentiostat interfaced to a PC for data acquisition via NOVA v1.10 (Metrohm, Netherlands). SPCEs were supplied by Gwent Electronic Materials Ltd (Pontypool, UK); the sensor is fabricated using carbon ink (C2030519P4) for the working electrode and a Ag/AgCl ink (C61003P7) for the reference electrode.

Reagents

Monosodium phosphate and disodium phosphate buffers were obtained from BDH Lab Supplies (Poole, UK). Deionised water was obtained from a Purite System (Purite Oxon., UK). Stock solutions of the phosphate buffers were individually prepared at a concentration of 0.2 M by dissolving the appropriate mass of solid in deionised water. These were then titrated to give the desired pH of buffer stock solution. Sodium chloride, sodium hydroxide, riboflavin and pyridoxine hydrochloride were purchased from Sigma Aldrich (Dorset, UK). Sodium chloride from was prepared to a concentration of 1.0 M by dissolving the appropriate mass in deionised water; this was added to the working standard to make a working solution 0.1 M sodium chloride solution. A primary stock solution of pyridoxine hydrochloride was prepared by dissolving the required mass in deionised water to give a concentration of 1 mg/mL. Sodium hydroxide was prepared to a concentration of 0.1 M by dissolving the appropriate mass in deionised water; a primary stock solution for riboflavin was prepared to a concentration of 1 mg/mL by dissolving the appropriate mass in 0.1 M sodium hydroxide. Working standards, for voltammetric studies, were prepared by dilution of the primary stock solution with either phosphate buffer or water to give a final concentration of 0.1 M phosphate buffer.

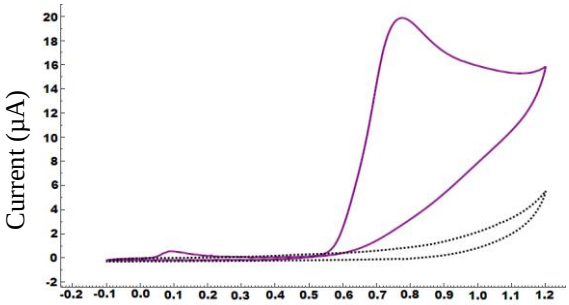
III. RESULTS AND DISCUSSION

Voltammetric Profiles

Most vitamins possess electrochemical activity and the voltage at which they produce detectable peaks is usually dependant of pH. The pH of meat post-mortem can vary significantly due to chemical changes brought about by stress and genetic traits [13]. Meat can have a Dry Firm and Dark (DFD) appearance with an abnormally high pH (> 6) [14] or conversely meat can have an abnormally low pH at 45 minutes post slaughter (< 6) which is termed Pale Soft and Exudative (PSE) [15]. A pH range of 5-7 was subsequently chosen to demonstrate the possibility of changing the peak position if interfering responses occur from the sample matrix.

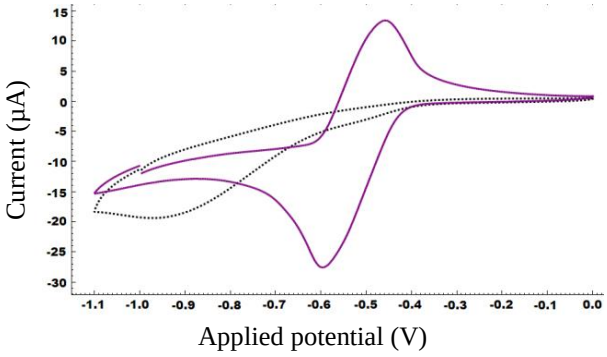
The cyclic voltammogram in figure 3 shows the SPCE response in the presence and absence of pyridoxine, on the anodic scan a well-defined oxidation peak is observed.

Figure 3. Cyclic voltammogram of pyridoxine hydrochloride at 0.2 mg/mL in pH 7 phosphate buffer. Scan rate 100 mV/s.



The cyclic voltammogram in figure 4 shows well-defined oxidation and reduction peaks for riboflavin at the plain SPCEs.

Figure 4. Cyclic voltammogram of riboflavin at at 0.4 mg/mL in pH 7 phosphate buffer. Scan rate 100 mV/s.

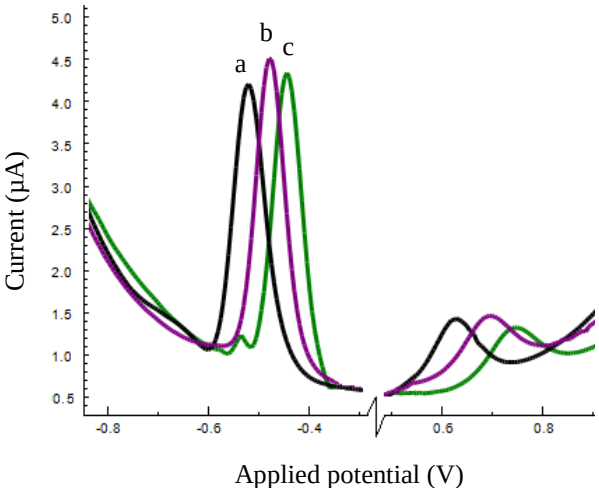


With both vitamins producing an oxidation peak a more sensitive technique, differential pulse voltammetry (DPV), was employed to measure both vitamins in a single anodic scan. The DPV in figure 5 shows the well-defined responses for both vitamins over the pH range 5-7. The peak potentials (Ep) of the two vitamins are separated by a wide potential window making their simultaneous determination feasible. The Ep of B_2 = -0.46 V at pH 6 vs Ag/AgCl and the Ep of B_6 = +0.70 V at pH 6 vs Ag/AgCl.

Calibration Studies

Calibration studies for riboflavin and pyridoxine were performed using the median pH outlined in the literature (pH 6). The peak currents for riboflavin were linearly correlated over the concentration range of 0.5-300 µg/mL (R_2=0.988) and pyridoxine has a linear range of 1.0-30 µg/mL (R_2=0.995). The sensitivities were 0.720 µA/µg/mL and 0.050 µA/µg/mL respectively. Replicate determinations of each concentration (*n=3*) gave a coefficient of variation of 6.94 % for riboflavin, and 3.91 % for pyridoxine.

Figure 5. Differential pulse voltammograms of a 10 µg/mL solution of riboflavin and pyridoxine at a) pH 7, b) pH 6, c) pH 5. Scan rate 50 mV/s



Analytical Applications

The SPCEs can achieve limits of detection in line with the low physiological concentrations reported for muscle tissue (Table 1) and were able to produce peak responses linearly over the pH range of interest. The application of these electrodes for the analysis of other food products and pharmaceutical preparations is currently underway with meat analysis to shortly follow.

IV. CONCLUSIONS

The successful voltammetric analysis of these water-soluble vitamins with un-modified screen-printed carbon electrodes provides a promising platform for the quantification of vitamins in meat products.

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