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Short communication

Development of a disposable pyruvate biosensor to determine pungency in onions (*Allium cepa* L.)

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Abstract

A disposable prototype pyruvate biosensor was constructed using pyruvate oxidase immobilised on mediated meldolas blue electrodes to determine pungency in onions (*Allium cepa* L.). The optimum operating potential was +150 mV (versus Ag/AgCl). A strong correlation between the biosensor response and untreated onion juice of known pyruvate concentration 2–12 μmol/g fresh weight (FW) was demonstrated. The biosensor was able to differentiate between low and high pungency onions. The detection limit using 1 unit of pyruvate oxidase was 1–2 μmol/g FW. Optimum concentrations of co-factors TPP, FAD and $MgSO_4$ comprising the enzyme cocktail were determined as being 0.04, 0.1 and 30 mM, respectively.

Keywords: Meldolas blue; Fresh produce; Quality assurance; Sweet onions

1. Introduction

Bulb onions are the second most important horticultural crop after tomatoes (Griffiths et al., 2002) and are consumed worldwide for their unique flavour. Increasingly, low pungency bulbs (often referred to colloquially as mild and/or sweet onions) are consumed raw in the USA and elsewhere. Pyruvate concentration (μmol/g FW) in macerated onion tissue is used as a quality assurance indicator of pungency (Schwimmer and Weston, 1961; Wall and Corgan, 1992; Crowther et al., 2005) or flavour intensity in most onion producing countries. Typically, low pungency onions have a pyruvate concentration of ca. <5 μmol/g FW and command a price premium.

Despite improvements to the original Schwimmer and Weston (1961) method over the last four decades, current quality assurance assays for onion pungency (e.g. Randle and Bussard, 1993; Yoo and Pike, 2001) are still relatively time-consuming and expensive. Confidence in the accuracy of pyruvate measurements is becoming more important, particularly as the popularity of low pungency onions increases (Yoo and Pike, 2001; Havey et al., 2002). Pungency tests are currently out-sourced. Decentralising the current pyruvate assay will empower growers and packers marketing low pungency onions to improve their quality assurance procedures.

The demand for reliable and inexpensive methods for the assessment of fresh produce quality is set to expand; biosensors offer a viable opportunity to fulfil this niche (Terry et al., 2005). Over the last 20 years, research has been carried out to produce a pyruvate biosensor, mainly for clinical applications (Table 1). The present study describes the development of an amperometric biosensor to detect and quantify the pyruvate concentration in juice from macerated onion tissue based on the following enzyme reaction:

$$\text{Pyruvate} + HPO_4^{2-} + O_2 \xrightarrow{\text{pyruvate oxidase (PyOx)}} \text{acetylphosphate} + H_2O_2 + CO_2$$

Mediators were used to reduce the effects of electrochemically active species, found in many food matrices (Terry et al., 2005). Meldolas blue was the preferred mediator used for this study, the reaction of which is as follows:

$$\text{Pyruvate} + HPO_4^{2-} + O_2 \rightarrow \text{acetylphosphate} + \text{PyOx}(FADH_2) \quad (1)$$

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Table 1
A selection of biosensor formats used to detect pyruvate

Enzyme(s)	Detection	Detection range	Construction format	Reference
PyOx[a]	H_2O_2 (V not stated)	1–10 mM	Chemical bonding. Polyazetidine prepolymer, nylon membrane	Mascini and Mazzei (1987)
PyOx	0.2–0.5 V	0.38–1.03 mM	Modified carbon, methylene green	Kulys et al. (1992)
PyOx	0.3 V	1 μM–1.8 mM	Electropolymerisation, conductive redox polymer, glassy carbon	Arai et al. (1999)
PyOx/HRP[b]	H_2O_2 (−0.05 V)	0.1–3 mM	Modified carbon, methylene green	Bergmann et al. (1999)
PyOx	H_2O_2 (+0.65 V)	5 μM–5 mM	Covalent attachment to polytyramine	Situmorang et al. (2002)

[a] Pyruvate oxidase.
[b] Horseradish peroxidase.

$$\mathrm{PyOx(FADH_2)} + \mathrm{MB^+} \rightarrow \mathrm{PyOx(FAD)} + \mathrm{MBH} + 2\mathrm{H^+} \quad (2)$$

$$\mathrm{MBH} \rightarrow \mathrm{MB^+} + \mathrm{H^+} + 2\mathrm{e^-} \quad (3)$$

2. Materials and methods

2.1. Reagents, standards and plant material

All of the chemicals used were of analytical grade. Pyruvate oxidase (E.C. 1.2.3.3.; PyOx) derived from *Pediococcus* spp., thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), hydrochloric acid (HCl) and 2,4-dinitrophenyl hydrazine (2,4-DNPH); pyruvic acid sodium salt ($C_3H_3O_3Na$), magnesium sulphate ($MgSO_4$), sodium hydroxide (NaOH) and trichloroacetic acid (TCA); potassium chloride (KCl), disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Sigma (Dorset, UK), Fisher Scientific Chemicals (Dorset, UK), and BDH. Ltd. (Leics., UK), respectively. All reagents were made up in reverse osmosis water. FAD and TPP co-factors were made up as 3 and 6 mM stock solutions, respectively, and stored at −20 °C until required. $MgSO_4$ was prepared as a 0.9 M stock solution and stored at 4 °C. Pyruvate oxidase solution was made up in the co-factor mix. Pyruvic acid sodium salt for deriving calibration standards was made up as a 5 mM stock solution and stored at 4 °C.

Commercially grown onion cvs. SupaSweet (SS1), Renate, Hyfort, Red Baron, UK Sturon, Crystal, Marimba and Spanish Pandero bulbs were donated by F.B. Parrish and Son (Beds., UK), Moulton Bulb Co. Ltd. (Lincs., UK) or Bedfordshire Growers Ltd. (Beds., UK).

2.2. Onion pyruvate analysis

Total pyruvate was measured according to Schwimmer and Weston (1961) and Crowther et al. (2005) with slight modifications. Whole onion bulbs were homogenised using a domestic blender (Braun, Type 4192, Spain) (Yoo and Pike, 1999). The juice was left to incubate for 1 h at room temperature. Aliquots (1.5 ml) were transferred to Eppendorf tubes and centrifuged at 16,060 × *g* (rotor 3325) for 10 min (Biofuge Pico, Kendro Laboratory Products, Germany). Some samples were subsequently stored at −20 °C prior to analysis. Juices were thawed at room temperature for 30 min and diluted 15-fold in deionised water. Filtrates (0.5 ml) were added to 1 ml aliquots of 0.0125% (v/v) (2,4-DNPH) in 2 M HCl and 1.5 ml deionised water in boiling tubes. The mixture was briefly vortexed and incubated at 37 °C for 10 min. Five millilitres of 0.6 M NaOH was added and the absorbance at 420 nm recorded (Camspec M501, Camspec Ltd., Cambs., UK). A standard curve to allow calculation of pyruvate concentrations from onion samples was produced by taking 10 ml of 5 mM pyruvic acid stock solution and diluting to 1 mM, followed by serial dilutions giving a concentration range of standards of 0.04–0.4 mM. Pyruvate concentrations (μmol/g FW) in onion were determined from the equation of the straight line on the standard curve.

2.3. Unmediated electrodes

Screen printed disposable plain carbon electrodes were manufactured by Cranfield University, Silsoe, UK. The electrodes comprised of a central carbon working electrode (10 mm^2), a counter electrode and an Ag/AgCl reference electrode. The electrodes were printed using a DEK 247 screen-printer (DEK Printing Machines Ltd., Dorset, UK). Sensors were connected to an Autolab workstation (Echochemi, Utrecht, The Netherlands) via custom-made electrical connectors (RS Components, Northhants., UK). The Autolab was controlled by the Autolab General Purpose Electrochemical System (GPES) software. Measurements were initially carried out at +800 mV at 21 °C. All experiments were undertaken in triplicate. All electrodes were only used once before disposal.

Initially, the response of unmediated carbon electrodes without enzyme and co-factors to onion cv. Renate juice was examined. Reagents included 50 mM sodium phosphate buffer, pH 6.9, and co-factor mix A—2 units PyOx, 0.2 mM TPP, 0.01 mM FAD and 10 mM $MgSO_4$ (final concentrations). The electrochemical response to increasing pyruvate concentrations in previously frozen undiluted onion juice was also compared against a calibration curve using the modified Schwimmer and Weston (1961) assay. All measurements were made by depositing 20 μl KCl electrolyte on the electrode surface, applying the potential, then allowing a steady-state current to be reached before adding 20 μl onion juice.

2.4. Mediated electrodes

Generic carbon, mediated with meldolas blue (C2030519D5, Gwent Electronic Materials Ltd., Gwent, UK), comprised the