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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 operating potential was +150 mV. 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 and FAD and $MgSO_4$ comprising the enzyme cocktail were determined as being 0.04 mM, 0.1 mM 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 twenty years research has been carried out to produce a pyruvate biosensor, mainly for clinical applications (Table 1). This 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:

$$\text{Pyruvate} + HPO^{-2}_4 + O_2 \rightarrow \text{acetylphosphate} + \text{PyOx}(FADH_2) \quad (1)$$

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

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

INSERT TABLE 1

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 mM and 6 mM stock solutions, respectively, and stored at -20°C until use. $MgSO_4$ 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 Ltd. (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 1h at room temperature. Aliquots (1.5 ml) were transferred to eppendorf tubes and centrifuged at 16060 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 ml 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 to 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 a 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 cofactors to onion cv. Renate juice was examined. Reagents included 50 mM sodium phosphate buffer pH 6.9 and cofactor 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. GEM, Gwent, UK) comprised the working electrodes (28 mm^2) and were screen printed with Ag/AgCl reference/counter electrodes onto a PVC substrate in a two electrode configuration. Electrochemical measurements were carried out using a PalmSense potentiostat (Palm Instruments BV, The Netherlands). Electrochemical measurements were carried out at either +150 mV or +200 mV at 21°C.

Enzyme immobilisation was achieved by depositing an enzyme cocktail containing PyOx, TPP, FAD and $MgSO_4$ made up in 0.1 M sodium phosphate buffer pH 5.7 onto the surface of the working electrode. Electrodes were left to air dry for 4h, then subsequently stored at 4°C prior to use. The optimum pH for hydrogen peroxide liberation from the reaction of pyruvate oxidase and pyruvate in the presence of phosphate and oxygen is 5.7. Irrespective of pyruvate content (2.3 – 6.6 µmol/g FW), the pH of onion juices for cvs. Red Baron, SS1, Marimba, Crystal, UK Sturon and Spanish Pandero marketed in the U.K. corresponded with the optimum for PyOx activity.

The electrochemical response to undiluted juices from individual onion cvs. SS1 and Renate bulbs of increasing pyruvate concentration were examined in two separate experiments using meldolas blue sensors.

2.6 Interference

The effect of operating potential on interference in juices from different onion (cvs. Red Baron, Spanish Pandero and Hyfort) cultivars were compared at +150 mV and +200 mV using cofactor mix B: 1 unit PyOx, 0.04 mM TPP, 0.1 mM FAD and 30 mM $MgSO_4$ (final concentrations).

3. **Results and discussion**

3.1 Response of unmediated carbon electrodes with and without PyOx and cofactors to onion juice

The electrochemical biosensor response to onion cv. Renate juice was considerably amplified with the addition of enzyme and co-factors (data not shown). However, a significant response was also evident with the addition of juice on bare electrodes due to other electrochemically active species in onion inevitably being oxidised at +800 mV (data not shown). Nevertheless, these preliminary results demonstrated the feasibility of measuring pyruvate concentration in onion juice using an amperometric biosensor format, but in order to reduce the biosensor operating potential, mediated sensors were adopted for further biosensor development.

3.2 Response of mediated meldolas blue electrodes to undiluted onion juice

Meldolas blue biosensors responded positively to increasing pyruvate concentrations in onion juice at a significantly reduced potential of +200 mV compared with unmediated electrodes at +800 mV. An operating potential of +200 mV was adopted following preliminary experiments where a constant operating range between +50 mV and +250 mV was demonstrated (data not shown). Furthermore, the meldolas blue biosensor gave considerably enhanced signals compared with the standard carbon format. A good correlation between the mediated biosensor responses to known pyruvate within juices of onion cvs. SS1 (low pungency) and Renate (high pungency) was demonstrated (Figs. 1 & 2).

INSERT FIGURE 1

INSERT FIGURE 2

3.3 Interference Experiments

There was, with some cultivars, a significant response on bare compared with enzyme immobilised mediated electrodes, indicating the presence of interference compounds. There was also a clear variation in background current across and within onion cultivars at +150 mV and +200 mV. This background response was reduced at +150 mV without any deterioration in performance. Levels of interference were 4.7% ± 1.8, 80.3% ± 5.5 and 32.0% ± 4.4 at +150 mV and 31.0% ± 3.8, 89.3% ± 1.9, 42.7% ± 8.1 at +200 mV for cvs. Red Baron, Spanish Pandero and Hyfort, respectively. The corresponding pyruvate contents ranged between 4.3 – 6.6, 7.7 – 9.5 and 10 – 10.7 μmol/g FW for cvs. Red Baron, Spanish Pandero and Hyfort, respectively. These results suggested that major polyphenols in red-skinned onions (cv. Red Baron; Price et al., 1997), were unlikely to be a major contributing factor given the low interference response.

4. Conclusion

Meldolas blue mediated electrodes were shown to be the best biosensor format tested for the amperometric detection of pyruvate in the juice from macerated onion tissue. There was a strong correlation between the biosensor response and known pyruvate concentrations (2 – 12 μmol/g FW) in onion as measured using the modified Schwimmer and Weston (1961) colorimetric assay. No sample dilution was necessary as found with some other pyruvate biosensors developed for clinical applications (*cf.* Table 1).

This preliminary study has demonstrated for the first time the possibility of replacing the standard colorimetric assay used ubiquitously by the world onion industry for determining pyruvate concentration with a more rapid method using a mediated amperometric biosensor. Introduction of a pyruvate biosensor for onions will empower growers to undertake their own quality control, rather than outsourcing pungency analysis. Ongoing work aims to further improve biosensor performance and elucidate possible variations in cultivar interference.

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References

Arai, G., Noma, T., Habu, H., Yasumori, I., 1999. Pyruvate sensor based on pyruvate oxidase immobilized in a poly(mercapto-p-benzoquinone) film. J. Electroanal. Chem. 464, 143-148.

Bergmann, W., Rudolph, R., Spohn, U., 1999. A bienzyme modified carbon paste electrode for amperometric detection of pyruvate. Anal. Chim. Acta 394, 233-241.

Crowther, T., Collin, H., Smith, B., Tomsett, B., O'Connor, D., Jones, M., 2005. Assessment of flavour of fresh uncooked onions by taste panels and analysis of flavour precursors, pyruvate and sugars. J. Sci. Food Agric. 85, 112-120.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., Smith, B., 2002. Onions-a global benefit to health. Phytother. Res. 16, 603-615.

Havey, M. J., Cantwell, M., Jones, M. J., Schmidt, N. E., Uhlig, J., Watson, J. F., Yoo, K. S., 2002. Significant variation exists among laboratories measuring onion bulb quality traits. HortScience 37, 1086-1087.

Kulys, J., Wang, L., Daugvilaite, N., 1992. Amperometric methylene green-mediated pyruvate electrode based on pyruvate oxidase entrapped in carbon paste. Anal. Chim. Acta 265, 15-20.

Mascini, M., Mazzei, F., 1987. Amperometric sensor for pyruvate with immobilized pyruvate oxidase. Anal. Chim. Acta 192, 9-16.

Price, K. R., Bacon, J. R., Rhodes, M. J. C., 1997. Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*). J. Agric. Food Chem. 45, 938-942.

Randle, W. M., Bussard, M. L., 1993. Streamlining onion pungency analyses. HortScience 28, 60.

Schwimmer, S. S., Weston, W. J., 1961. Enzymatic development of pyruvic acid in onion as a measure of pungency. J. Agric. Food Chem. 9, 301-304.

Situmorang, M., Gooding, J. J., Hibbert, D. B., Barnett, D., 2002. The development of a pyruvate biosensor using electrodeposited polytyramine. Electroanalysis 14, 17-21.

Terry, L. A., White, S. F., Tigwell, L. J., 2005. The application of biosensors to fresh produce and the wider food industry. J. Agric. Food Chem. 53, 1309-1316.

Wall, M. M., Corgan, J. N., 1992. Relationship between pyruvate analysis and flavour perception for onion pungency determination. HortScience 27, 1029-1030.

Yoo, K. S., Pike, L. M., 1999. Development of an automated system for pyruvic acid analysis in onion breeding. Sci. Hort. 82, 193-201.

Yoo, K. S., Pike, L. M., 2001. Determination of background pyruvic acid concentrations in onions, Allium species, and other vegetables. Sci. Hort. 89, 249-256.

List of Figures and Tables

Table 1.

A selection of biosensor formats used to detect pyruvate

Enzyme(s)	**Detection**	**Detection Range**	**Construction Format**	**Reference**
PyOx[1]	H_2O_2 (V not stated)	1-10 mM	Chemical bonding. Polyazetidine prepolymer, nylon membrane	Mascini and Mazzei, 1987
PyOx	0.2-0.5V	0.38-1.03 mM	Modified carbon, Methylene green	Kulys et al., 1992
PyOx	0.3V	1 μM-1.8 mM	Electropolymerisation, conductive redox polymer, glassy carbon	Arai et al., 1999
PyOx/HRP[2]	H_2O_2 (-0.05V)	0.1-3 mM	Modified carbon , Methylene green	Bergmann et al., 1999
PyOx	H_2O_2 (+0.65V)	5 μM-5 mM	Covalent attachment to polytyramine	Situmorang et al., 2002

[1] Pyruvate oxidase; [2] Horseradish peroxidase

Figure 1

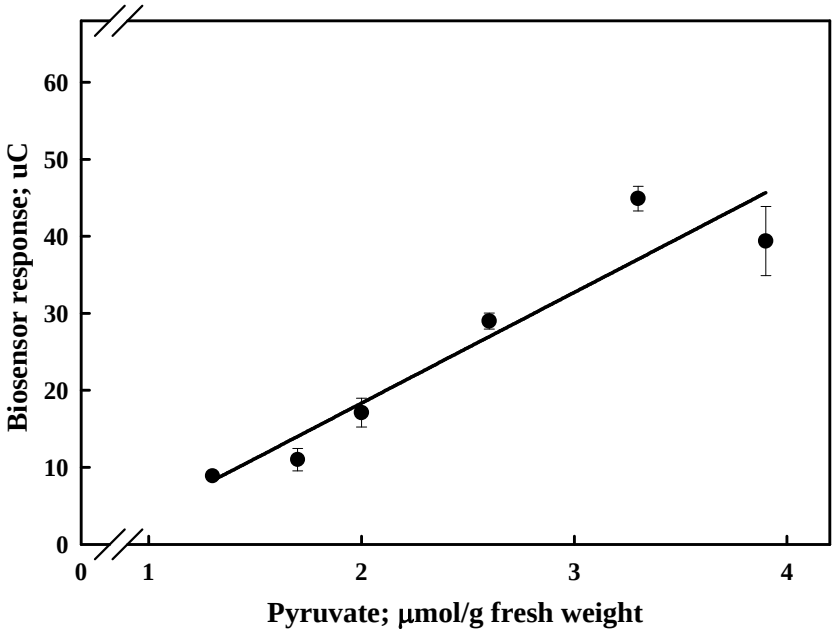

Figure 2