

Development of a Glucose Biosensor for Rapid Assessment of Strawberry Quality: Relationship between Biosensor Response and Fruit Composition

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Sugars are intimately related to the taste of strawberry fruit and therefore to quality. A disposable prototype glucose biosensor was constructed using glucose oxidase (GOx) immobilized onto Meldolas Blue mediated screen printed electrodes, to determine glucose content in diluted strawberry juices. Several experimental variables that affect biosensor performance, such as applied potential, GOx loading, and pH of the buffer/electrolyte solution were optimized by means of a 2^3 central composite design. Optimum applied potential, pH, and enzyme loading were +300 mV (versus Ag/AgCl), 7.2, and 20 U, respectively. Although the linear range (0–10 mM) was not significantly affected by the optimization process, the signal given by the biosensor was increased by as much as 3-fold as compared to preliminary experiments, and the reproducibility of the measurements was improved. Unlike total soluble solids (TSS), and as hypothesized, the constructed GOx biosensor was able to discriminate and rank eight different strawberry cultivars on the basis of their glucose content when compared to known concentrations measured by standard HPLC. A detailed study of the possible interferences (viz. total phenolics, antioxidant capacity, and organic acids) found in strawberry samples that could affect biosensor performance was also performed to further understand the relationship between biosensor response and sample composition. Under the imposed experimental conditions the constructed biosensor acted interference-free.

KEYWORDS: Antioxidant capacity; organic acids; sugars; total phenolics; TSS

INTRODUCTION

The compositional analysis of fruit and vegetables is extremely important for defining eating quality of fresh produce. In strawberry fruit, nonstructural carbohydrates, including glucose, fructose, and sucrose, account for more than 80% of the total soluble solids (1, 2) and are intimately related to taste (2, 3). In this context, perceived sweetness is directly related to sugar concentration in strawberry fruit and may be used as an index of consumer acceptability (4). However, sugar concentration is often confused with total soluble solids (TSS) as determined by refractometry. TSS is one of the parameters ubiquitously used during standard quality control (QC) of strawberry fruit, yet it has been shown that TSS is often poorly correlated with sugar concentration in strawberry (3) and some other horticultural crops (5–7). More accurate techniques such high performance liquid chromatography (HPLC) (2, 3), gas chromatography (GC) (8), or enzymatic test kits (9) are available for quantifying sugars; however, they tend to be time-consuming and costly; hence, they are not appropriate to be routinely applied in the fresh produce industry for standard daily QC.

The development of analytical methods that are low cost, easy to operate, and portable would be of great importance for berry fruit in particular and indeed for the overall fresh produce

industry. In this perspective, biosensors may fulfill most of the above-mentioned requirements and therefore may provide the food industry with a promising alternative to standard QC, not only by enhancing the relevance and extent of tests carried out but also by measuring specific analytes which are key indicators of produce quality and consumer acceptability (10). Biosensors for glucose determination using GOx have already been applied to some other fresh produce types or beverages (11–16); this said, most of these have not yet been commercialized due, in part, to the high variability and complexity of the food or fruit juice matrices. In this context, strawberries are not an exception since great variability exists in the composition of fruits of different cultivars (cvs.) (17) or from fruit derived from plants grown under different agronomic conditions (2, 18, 19). Moreover, strawberry fruits are rich sources of electrochemically active compounds (viz. ascorbate (AsA), phenolic acids, and anthocyanins (2, 20, 21)) which indeed may easily undergo oxidation or reduction at the surface of the electrode under relatively low operating potentials (10, 14). Accordingly, the aim of the present study was to develop and optimize, using experimental design methodology (15), a new amperometric GOx-based biosensor for rapid quantification of glucose in strawberry juice. Screen-printed carbon electrodes (SPCE), mediated with Meldolas Blue (MaB+), were chosen based on previous work carried out by Abayomi (16) and because of the known versatility of this mediator toward both oxygenase and dehydrogenase enzyme

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formats (22–25). Finally, the applicability of the constructed GOx-based biosensor was tested against eight different strawberry cultivars, previously characterized for their content in main sugars, organic acids, total phenolics, and antioxidant activity, to further understand the relationship between the biosensor response and sample composition.

MATERIALS AND METHODS

Biosensor Development and Optimization. *Reagents.* All reagents including glucose oxidase (EC 1.1.3.4; GOx) derived from *Aspergillus niger*, were purchased from Sigma (Dorset, U.K.) unless otherwise stated. The chemicals used were of analytical grade, and solutions were prepared with Milli-Q water (Millipore Inc.; $\sigma = 18 \text{ M } \Omega \text{ cm}^{-1}$). Stock solutions of glucose (10 mM) were prepared daily in 0.1 M sodium phosphate buffer at optimum pH and left to equilibrate for ca. 4 h before measurements started.

Screen-Printed Electrodes and Instrumentation. Screen-printed electrodes (SPE) (C2030519D5, Gwent Electronic Materials Ltd. GEM, Gwent, U.K.) were used for all measurements (24, 25). The electrodes were screen-printed in a two electrode configuration comprising a generic carbon mediated with MaB+ working electrode (28 mm^2) and a combined Ag/AgCl reference/counter electrodes onto a PVC substrate. All electrochemical measurements were performed using a Palmsens potentiostat (Palm Instruments BV, The Netherlands). Results from these experiments were processed using Ivium Palmsens PC software.

Biosensor Optimization. The amperometric response (current density) obtained with an enzyme-based biosensor is a function of several experimental variables including applied potential (E_{ap}), units of enzyme loading (C_{GOx}), pH of the buffer solution, and concentration of the mediator. In this context, E_{ap} , C_{GOx} , and pH were optimized by means of a 2^3 central composite design (CCD), using standard glucose solutions (10 mM) prepared in 0.1 M sodium phosphate buffer and adjusted to optimum pH (15). In the present study, selected levels for each variable to be optimized were chosen corresponding to the plus (+), minus (−), and central (0) points and based on results obtained during preliminary experiments (E_{ap} , 300, −200, and 50 mV; pH, 8, 4 and 6; C_{GOx} , 15, 5, and 10 U, respectively).

Enzyme immobilization was achieved by simply depositing a known volume of GOx solution made up in 0.1 M sodium phosphate buffer at pH 5.7 onto the surface of the working electrode (24, 25). Electrodes were then left to air-dry for 4 h at room temperature and subsequently stored at 4 °C overnight until used the following day.

Biosensor Calibration and Selectivity. Prior to the analysis of real samples, the GOx-based biosensor was calibrated against glucose standard solutions ranging from 0 to 50 mM made up in 0.1 M phosphate and adjusted to optimum pH values. The selectivity of the biosensor in the presence of other sugars found in strawberry (viz. fructose and sucrose) extracts was also assessed by adding known concentrations (0, 5, 10, and 20 mM) of the above-mentioned sugars to the glucose standard solutions.

Characterization of Strawberry Fruits. *Plant Materials.* Eight different strawberry cultivars (viz. Christine, Elsanta, Flamenco, Florence, Jubilee, Sonata, Symphony, and Pearl) were selected according to individual differences in sugar/acid taste profiles (Duncalfe, H., personal communication). Plants were grown under standard commercial practices and supplied by H&H Duncalfe (Cambs., U.K.) or other local suppliers.

Total Soluble Solids (TSS), Objective Color, and Sample Preparation. Upon arrival at the laboratory, the objective color of 10 fruits per cv. was measured using a Minolta DP-400 colorimeter (Minolta Co. Ltd., Japan) with an 8 mm light-path aperture (26). The instrument was calibrated with a Minolta standard white tile CR-400 ($Y = 93.5$, $x = 0.3114$, $y = 0.3190$). The mean of three readings at 3 equidistant points around the equatorial axis was recorded and the lightness (L^*), chroma (color saturation; C^*), and hue angle (H°) automatically calculated (26). In addition, TSS was measured for each cultivar (cv.) in triplicate using a digital Palette PR-32 α refractometer (Atago, Japan). Afterward, samples (ca. 1 kg) were immediately snap-frozen in liquid nitrogen and kept at −40 °C until subsequent analysis. A portion of the fresh-frozen samples was directly used for biosensor measurement and sugar determination by HPLC on a fresh weight (FW) basis, whereas another 150 g for each cv.

was freeze-dried in an Edwards Modulyo freeze drier (W. Sussex, U.K.) for 5 days at 0.015 kPa. Lyophilized samples were then ground using a pestle and mortar to a fine powder, weighed, and returned to the freezer (−40 °C) prior to being used for additional sugar and organic acid determinations by HPLC, total phenolics (TP), and antioxidant capacity (AC) measurements on a dry weight (DW) basis.

HPLC Determination of Sugars and Organic Acids. Sugar determination on a FW basis was performed on diluted strawberry homogenates (1:20 (w/v) in phosphate buffer at optimum pH), whereas extraction and quantification of both sugar and organic acid on a DW basis was performed as reported previously for strawberry and other soft fruits (2, 6).

Determination of Total Phenolics and Antioxidant Capacity. Total phenolics (TP) were extracted by dissolving 150 mg of freeze-dried powder in 3 mL of 80% (v/v) aqueous ethanol solvent and held in a water-bath for 2 h at 70 °C, mixing every 20 min. The solution obtained was filtered as previously described and the clear filtrate analyzed according to the Folin–Ciocalteu method (FCM) (2). Antioxidant capacity (AC) was also measured on freeze-dried samples using the ferric reducing ability of plasma (FRAP) (27) assay as described previously (2).

Electrochemical Measurements. Amperograms were obtained by depositing 20 μL of electrolyte solution (0.1 M KCl in the previously described sodium phosphate buffer) on the electrode surface, applying the optimum potential (+300 mV) and allowing a steady current to be reached ($t = 100 \text{ s}$) before depositing 20 μL of standard or diluted (1:20; w/v) strawberry homogenate (24, 25). A total time of 200 s was required for each amperometric measurement. Finally, the electrochemical response to different glucose concentrations in strawberry juices was compared against glucose content determined by HPLC.

Data Analysis. Data analysis including CCD was carried out using Statgraphics centurion (trial version XV) and statistical analysis using Genstat for Windows version 9.1.0.147 (VSN International Ltd., Herts., U.K.). Least significant difference values (LSD; $P = 0.05$) were calculated for mean separation using critical values of t for two-tailed tests. Variations among biosensor responses or main treatments were plotted in SigmaPlot 9.0 (Systat Software, Inc., London, U.K.). Tests for correlations between mean values for analyte concentrations were made using Spearman's Rank Correlation. Correlations are presented with the Spearman's correlation coefficient (r) and P value based on a two-tailed test.

RESULTS AND DISCUSSION

Optimization of the Biosensor Response. E_{ap} , pH of the buffer/electrolyte solution, and units of enzyme loading (C_{GOx}) in the enzyme cocktail and subsequently deposited onto the surface of the working electrode were optimized by means of experimental design methodology, using a central composite design (CCD- 2^3). The use of similar approaches for the optimization of several parameters that may affect sensor or biosensor responses has satisfactorily been applied in the past (15, 28, 29). CCD encompasses numerous advantages, as compared to more traditional approaches (30) since it allows optimization of each variable simultaneously and considers the influence of the interaction between variables in the final biosensor response. Analysis of variance (ANOVA) of the optimization procedure revealed that neither enzyme loading (C_{GOx}), within the range studied, nor the interactions between this variable and pH or E_{ap} significantly affected the signal given by the biosensor. In contrast, E_{ap} ($P = 0.005$) and pH of the buffer solution ($P = 0.007$) were crucial variables in terms of maximizing the biosensor response (current density). The P value for the lack-of-fit ($P = 0.055$) was higher than 0.05; therefore, the quadratic model proposed by the optimization process ($I (\mu\text{A}) = 0.315 - 21.763 \cdot E_{\text{ap}} - 0.45 \cdot \text{pH} + 3.406 \cdot E_{\text{ap}}^2 + 5.004 \cdot E_{\text{ap}} \cdot \text{pH} + 0.113 \cdot \text{pH}^2$), excluding nonsignificant variables, was appropriate for the experimental data at the 95% confidence level. The model clearly reflected that, within the range tested, greater pH and applied potential values resulted in greater current densities. Given the information provided by

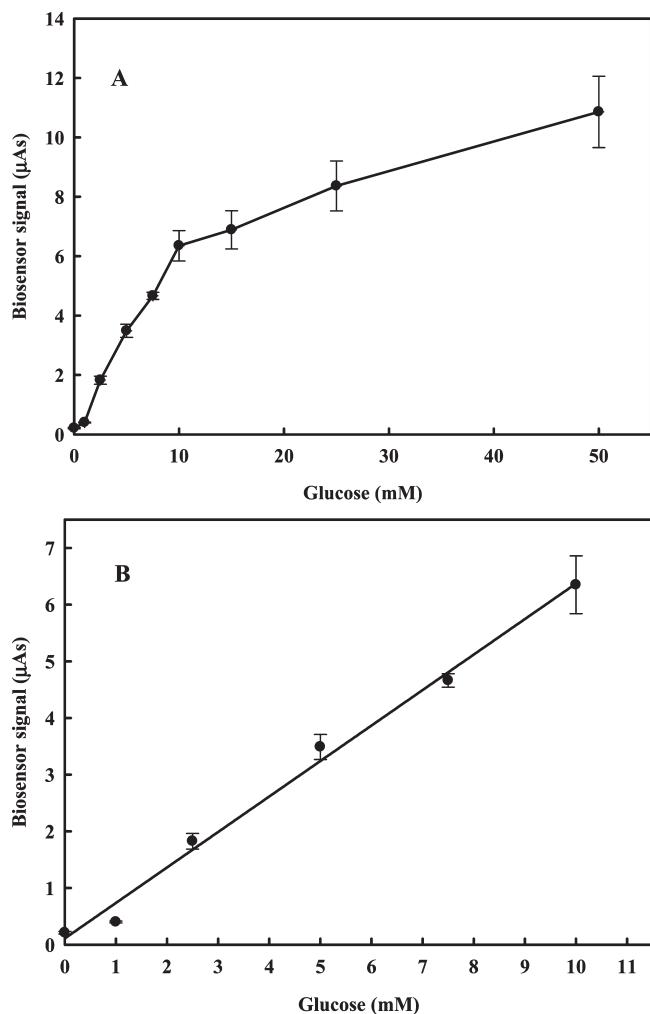


Figure 1. Calibration of GOx-mediated biosensor using glucose standards in phosphate buffer at pH 7.2. E_{ap} = +300 mV (vs Ag/AgCl); 10 units of GOx. Standard deviation bars are from the mean of 5 measurements. (A) Biosensor response up to glucose concentrations of 50 mM. (B) Biosensor response within the linear range (up to 10 mM glucose), (μAs) = $0.626 \times$ glucose concentration (mM) + 0.110; $P < 0.001$. $R^2 = 0.99$.

the optimization process and on the basis of the results obtained during preliminary experiments, optimum values were fixed at +300 mV (vs Ag/AgCl), pH of 7.2, and $C_{GOx} = 20$ U. The use of higher potentials may have increased the level of interference caused by certain electroactive compounds, easily oxidized and commonly found in fresh produce matrices (10, 24). Moreover, pH values over 8 may have seriously affected the stability of the enzyme (31).

Biosensor Selectivity and Linear Range. The GOx-based biosensor responded positively to increasing concentrations of glucose in 0.1 M phosphate buffer (pH 7.2) at an applied potential of +300 mV (versus Ag/AgCl). Although the linear range, up to 10 mM, was not affected by the optimization process, the signal given by the biosensor (μAs) = $0.626 \times$ glucose concentration (mM) + 0.110 (Figure 1) was increased by as much as 3-fold in comparison with that in preliminary experiments, and the reproducibility of the signal was considerably improved (data not shown). Although using other electrode formats, others have reported similar range of concentrations when developing glucose sensors for food applications (13, 14), the lower limit of detection using 20 U of GOx was equivalent to ca. 0.01 mg glucose g^{-1} FW. In agreement with previous studies, using similar SPC electrode

Table 1. Variations in Weight (W) Characteristics, Total Soluble Solids, and Objective Color (a) of Eight UK-Grown Strawberry Cultivars

cultivar	W (g)	TSS (%)	L*	C*	H°
Christine	16.13	9.53	44.01	54.83	37.68
Elsanta	17.49	9.17	37.31	45.32	36.97
Flamenco	22.70	11.63	44.44	54.95	54.95
Florence	21.12	8.87	31.79	43.74	29.76
Jubilee	23.09	9.17	39.53	50.82	34.92
Pearl	23.68	9.27	35.94	46.48	46.48
Sonata	21.01	9.10	34.03	43.72	32.99
Symphony	15.49	8.57	38.17	50.18	37.58
Mean	20.09	9.41	38.15	48.75	38.91
LSD ($P < 0.05$)	5.422	0.171	3.479	6.521	3.427

^a L* is lightness, C* is chroma, and H° is hue angle.

configurations (16), virtually no response was obtained with the addition of fructose and sucrose in solutions, confirming the selectivity of the GOx-based biosensor toward the glucose substrate.

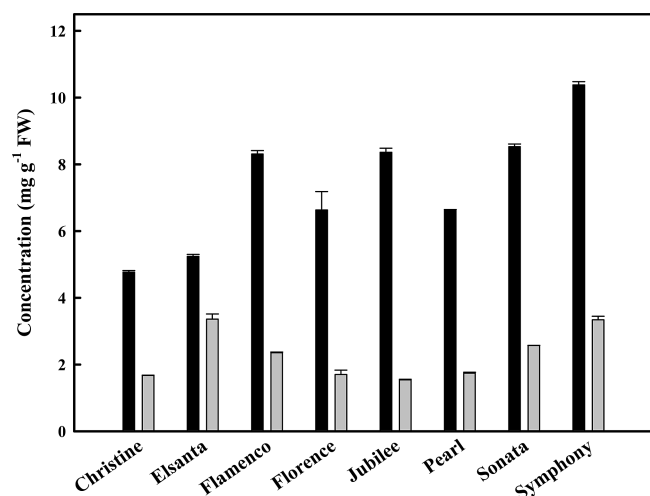
Characterization of Strawberry Fruits. Characterization of potential biosensor interferences and target components in the different strawberry cultivars analyzed was achieved by previously reported methods (2, 6). Overall, values for all target analytes differed significantly between cultivars but were in the range of those found by others (2–4, 17, 18). Accordingly, variations in taste and health-related components of fruits from different cultivars (6, 17) or from plants grown under different conditions (2, 4, 18) have been extensively reported in strawberry or other soft fruits. From the different cultivars analyzed, berry weight ranged from 15.49 to 23.68 g and significantly differed between cultivars; smaller fruits were obtained from cvs. Christine and Symphony, whereas fruits from the cv. Jubilee were the larger fruits and had greater dry matter as a proportion of fresh weight (14.1 g DW 100 g^{-1} FW) (Table 1).

Objective color of fruits from different cultivars was significantly different (Table 1) and hence in agreement with that shown by others (32, 33). Lower H° indicates higher redness, and accordingly, fruits from cv. Florence (H° = 29.76) had deeper red coloration than the rest of the cultivars analyzed (H°_{mean} = 38.91). Similar objective color values were recorded for cvs. Flamenco and Christine with fruits characterized by having high chroma and lightness values (C* = 54 and L* = 44, respectively).

In strawberry fruits, nonstructural carbohydrates are the main components of dry matter. In agreement with that previously reported (2, 4, 34), results showed that sugars accounted for 6 to 8% of the total fresh matter. Fructose, glucose, and sucrose were the three main sugars found in the different strawberry cultivars investigated. Both total and individual sugar values differed between cultivars (Table 2). Strawberry cv. Jubilee fruit had as much as 1.3-fold higher sugar content than the mean value of the other cultivars analyzed. Both glucose and fructose were present in all cultivars at similar proportions (ca. 1:1); glucose content ranged from 15.30 to 29.74 mg g^{-1} FW, whereas fructose varied from 17.21 to 32.06 mg g^{-1} FW, depending on the type of extraction used. In this context, sugar concentration was measured from either directly diluted (1:20; w/v) fruits or from the methanol-based extracts as described by Terry et al. (2). In all cases, sugar content was greater in those samples extracted with aqueous methanol as compared to water (Table 2). Glucose, fructose, and sucrose concentrations were on average 1.3-, 1.1-, and 1.5-fold greater in the methanolic extracts than in the directly measured strawberry juice. This said, sugar content was affected not only by the extraction used but also by the interaction between extraction method and cultivar. Indeed the use of a methanol-based solvent was more efficient in extracting sugars in

Table 2. Effect of Extraction Procedure (viz. Direct Determination on Diluted 1:20 (w/v) Strawberry Homogenates (Aqueous) or Methanol-Based Extraction As Described by Terry et al. (2007) (MeOH)) on Measured Sugar Concentration (mg g^{-1} FW) and the Sugar/Acid Ratio of Eight U.K.-Grown Strawberry Cultivars

cultivar	sucrose		glucose		fructose		total		sugar/acid	
	aqueous	MeOH	aqueous	MeOH	aqueous	MeOH	aqueous	MeOH	aqueous	MeOH
Christine	17.97	27.05	17.53	21.74	24.06	32.11	59.56	85.89	4.71	7.02
Elsanta	30.48	35.72	18.74	26.71	20.81	27.53	70.03	89.93	7.48	9.60
Flamenco	19.19	37.00	17.83	22.41	20.98	23.12	58.00	82.51	5.56	7.92
Florence	10.43	23.79	19.98	22.10	24.76	22.82	55.17	68.69	7.47	9.31
Jubilee	28.35	42.23	22.59	27.77	28.37	29.71	79.31	99.70	7.23	9.31
Pearl	28.65	34.32	15.69	20.01	17.96	23.48	62.30	79.73	6.12	7.92
Sonata	18.67	21.54	15.30	23.11	19.34	25.23	53.31	69.85	3.45	4.52
Symphony	10.82	22.06	18.34	22.68	21.68	23.84	50.84	68.54	3.32	4.47
mean	20.57	30.46	18.25	23.32	22.15	25.98	61.07	80.61	5.67	7.48
LSD ($P < 0.05$)	1.147		1.002		1.577		2.710		0.498	

**Figure 2.** Organic acid content (citric (black bars) and malic (gray bars)) from eight different strawberry cultivars. $\text{LSD}_{\text{Citric}}$ ($P = 0.03$) = 0.37; $\text{LSD}_{\text{Malic}}$ ($P = 0.01$) = 0.142. Values are the average of 3 measurements $\pm$ standard deviation (SD).

certain cultivars (viz. Christine, Flamenco, and Jubilee) than in others, indicating that measured sugar content was not only dependent on the extraction used but also on the sample matrix. As a result of the differences in the amount of sugars extracted between the different methodologies used, the sugar/acid ratio was significantly greater for samples extracted with methanol (7.5) than for those directly measured from diluted homogenates (5.7; relative units) (Table 2). Concomitant to this, Terry et al. (2) found greater amounts of sucrose as compared to the earlier values reported in the literature for strawberry fruits (3, 4, 18, 19). Fructose and glucose are nearly twice as soluble in methanol-based than in aqueous-based solvents, while sucrose is almost three times more soluble (35), and therefore, a short methanol-based extraction may have enhanced the solubility of these sugars, especially sucrose, into the extraction solution. Implications from these findings revealed that not only the type of extraction but also the solvent used and the matrix effect are key factors for accurate investigation of sugars in strawberry fruits. Similarly, Davis et al. (35) found that not only the total sugar content was affected but also the ratio of glucose, fructose, and sucrose within and across different onion cultivars was dependent on the extraction procedure. Consequently, comparison of sugar content in strawberry fruits from different studies should be treated with some caution.

Taste in strawberry fruits is, however, not just influenced by sugars. Acids and volatile compounds are also important contributors to strawberry taste and flavor, respectively (34). Among

the acids found in the different strawberry cultivars, citric, malic, and AsA acids accounted for ca. 62%, 19.5%, and 4%, respectively, of the total organic acids content and differed significantly between cultivars (Figures 2 and 3). Greater amounts of citric and malic were found in cv. Symphony (10.38 and 3.34 mg g^{-1} FW, respectively) (Figure 2), whereas highest ascorbic acid levels were found in cv. Flamenco (0.75 mg g^{-1} FW) (Figure 3). In addition to AsA, other electrochemically active compounds present in strawberry fruits were measured, and TP values ranged from 1.51 to 3.34 mg GAE g^{-1} FW with cvs. Christine and Flamenco having the highest values (Figure 3). Antioxidant capacity of strawberry fruits as measured by the FRAP assay also differed between cultivars, and in this case, cv. Florence (17.63 ± 1.14 $\mu\text{mol Fe}^{2+} \text{g}^{-1}$ FW) and cv. Symphony (15.17 ± 0.79 $\mu\text{mol Fe}^{2+} \text{g}^{-1}$ FW) had the greatest antioxidant capacity (Figure 3). No significant correlations were observed between TP and AsA.

GOx-Based Biosensor Performance with Strawberry Samples.

After optimization of biosensor performance and characterization of the different samples, the response of the biosensor and the associated background signal was tested by challenging the biosensor with serial dilutions of two strawberry samples (cvs. Elsanta and Symphony). Results showed that a dilution of 1:20 (w/v) was best for minimizing the background signal, as represented by the standard deviation, given by each sample (Figure 4). Then, the constructed GOx-based biosensor was tested under optimum experimental conditions against strawberry juices from eight cultivars with known differences in their sugar and acid profile. Results indicated that, although the glucose concentrations determined using the biosensor were significantly lower than those obtained using HPLC, there was a strong positive correlation ($R^2 = 0.73$; $P < 0.001$) (Figure 5) between the signal given by the biosensor and known concentrations of glucose in strawberry juice, regardless of the type of extraction used. In contrast, no correlations were found between TSS values and known glucose concentrations or total sugars when the same samples were analyzed (Figure 5). As already reported for strawberry (3, 10) and other horticultural crops (5–7), TSS values did not satisfactorily discriminate between those cultivars having high or low sugar content. For instance, cv. Jubilee which had as much as 1.4-fold higher glucose or 1.5-fold greater total sugar content than cv. Sonata (Table 2) had a similar TSS value. Accordingly, unlike TSS, the constructed GOx biosensor was able to discriminate and rank different cultivars based on their glucose content when compared to known concentrations measured by HPLC. Lower glucose concentrations as determined by the biosensor are not unusual, and therefore, a correction factor (ca. 2-fold) could be adopted. Discrepancies in the glucose content between both methods could be either explained by the specificity of the enzyme toward the β -D-glucose anomer (13, 31) or by a simple matrix effect. Glucose in fruits is present in both its α and β forms (13).

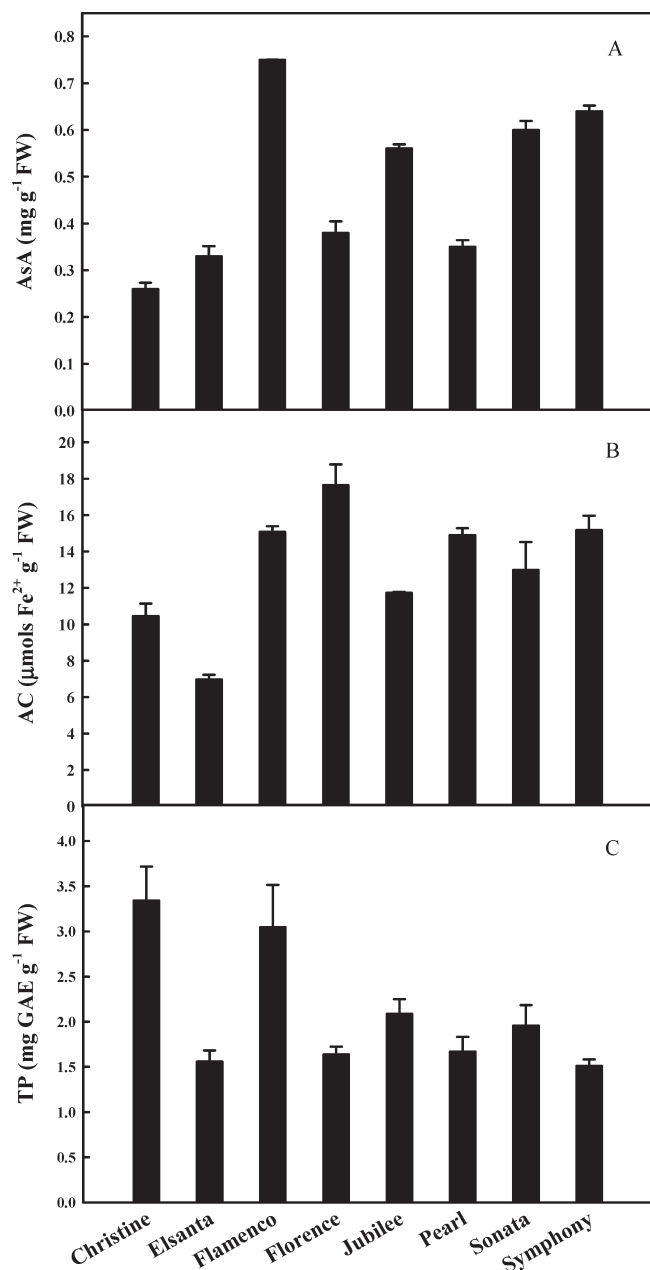


Figure 3. Characterization of possible biosensor interferences from eight different strawberry cultivars. (A) Ascorbic acid (mg g^{-1} FW), (B) antioxidant activity as measured by the FRAP assay ($\mu\text{mols Fe}^{2+} \text{g}^{-1}$ FW), and (C) total phenolics measured by the Folin–Ciocalteu assay (mg GAE g^{-1} FW). $\text{LSD}_{\text{AsA}} (P=0.03) = 0.027$; $\text{LSD}_{\text{AC}} (P=0.01) = 1.369$; $\text{LSD}_{\text{TP}} (P=0.01) = 0.429$. Values are the average of 3 measurements $\pm$ SD.

Recently, Kwack et al. (36) reported almost a one to one ratio between both anomers in strawberry fruits (cv. Maehyang); this said, no information is available about variations in this ratio between other cultivars. In other fruits such as pineapple, the percentage of α -D-glucose was 5 times greater than that of β -D-glucose (37). Jawaheer et al. (13) reported on the incorporation of mutarotase enzyme within the enzyme cocktail to measure total D-glucose in different tropical fruits. In the work described herein, the different steps involved in the preparation of the electrode enzyme were kept as simple as possible, and accordingly, we decided not to include the mutarotase enzyme into the cocktail mixture. Since the ratio between glucose and fructose (ca. 1: 1) in strawberry fruit seems to be consistent regardless of the cultivar or agronomic conditions in which the plants are grown (2, 4),

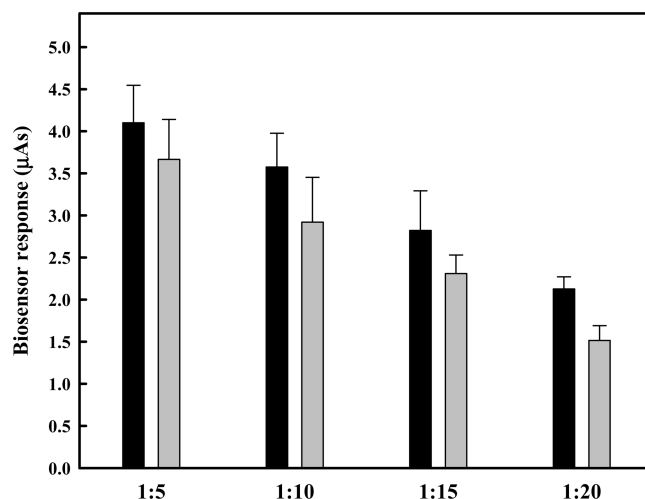


Figure 4. Effect of serial dilutions (5, 10, 15, and 20; w/v) of strawberry homogenates (cvs. Elsanta (black bars) and Symphony (gray bars)) in 0.1 M sodium phosphate buffer (pH 7.2) on the signal and related background noise given by the biosensor operating under optimum conditions. Values are the average of 5 measurements $\pm$ SD.

determination of glucose by means of the biosensor described herein would not only reflect glucose content but also could be adopted as an estimative measure of fructose content and therefore perhaps perceived sweetness in strawberry fruits.

One of the main challenges when designing a biosensor for food applications is that food samples contain certain compounds, different from the target analyte, that may easily undergo oxidation or reduction at the electrode surface under the selected operating potential (10, 14). Such electrochemically active compounds are known to be present in strawberry fruits in relatively high concentrations (viz. AsA (2), anthocyanins, and other phenolic compounds (2, 20, 21, 38)). Indeed, AsA and polyphenols are reducing agents, and their electrochemical properties are due to their ability to donate electrons (39). When developing biosensors for fresh produce, previous knowledge of the sample is required in order to fully understand the relationship between the biosensor response and sample composition. In the present study, total phenolics and antioxidant capacities of the fruits were measured by means of the already established Folin–Ciocalteu and FRAP assays, respectively. Both assays may give an overall indication of the concentration of electrochemically active compounds present in the different cultivars analyzed since both assays are based on radical or electron scavenging capacities (40). For instance, the FRAP assay is reliant on the ability of the antioxidants to reduce a ferric complex to a ferrous form (27). Aaby et al. (39) and Piljac-Zegarac et al. (41) have already shown there to be a positive correlation between results obtained by electrochemical measurements and those obtained by radical or electron scavenging assays in different standard or fruit solutions. Values for TP and AC described herein were in agreement with those found in the literature (2, 17, 19). No correlation was found between the GOx-based biosensor signal and either TP or FRAP values, indicating that under the conditions imposed in this study the biosensor acted free of interference. This said, cultivars having high amounts of AsA (Figure 3) (viz. Florence and Flamenco) gave higher variability when tested with the biosensor, which may have been due to a matrix effect (Figure 5). For the successful application of the developed biosensor, several strategies were undertaken through this work to reduce the possible number of interferences. First, the SPCE were mediated with MaB+ on the basis of previous studies (16, 24, 25) and due to the

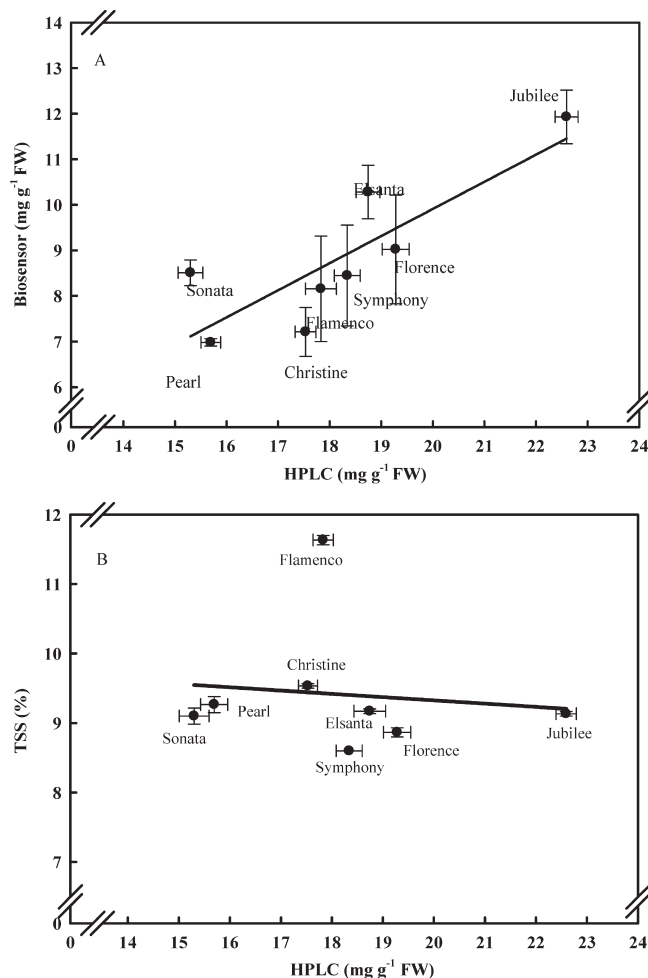


Figure 5. (A) GOx-based biosensor response to diluted strawberry homogenates (1:20; w/v) from eight different strawberry cultivars (viz. Christine, Elsanta, Flamenco, Florence, Jubilee, Sonata, Symphony, and Pearl) verified against HPLC determination. $R^2 = 0.732$; $P < 0.001$. (B) No correlation was observed between glucose or total sugar content and TSS values.

known versatility of this mediator toward both oxygenase and dehydrogenase enzyme formats (23). Mediators are used in biosensor technology to replace O_2 as an electron acceptor and allow biosensor performance at much lower operating potentials, hence limiting possible interferences caused by other electrochemically active species found in many food matrices (10). Second, as described earlier (Figure 4), by performing a 1:20 dilution of the strawberry homogenate, the background signal given by the samples was minimized considerably. Similar approaches were undertaken by others (14, 24, 25) when designing biosensors for the detection of target analytes in wine or onions.

Generally, results from this study have provided further evidence for the existent variability in the composition of strawberry fruits from different cultivars commonly found in the U.K. market. In addition, and for the first time, it has been demonstrated that a GOx-based biosensor could be used to measure glucose in strawberry fruits and therefore provide growers and retailers with a promising alternative to TSS, thus improving quality control (10). Analysis time was reduced by 40-fold as compared to that in conventional HPLC, and therefore, the GOx-based biosensor could be used for screening of large data sets in breeding programs. The proposed prototype biosensor would enhance the relevance of the analysis carried out by measuring specific analytes which are key indicators of strawberry quality

and consumer acceptability. Future research will aim at adding functionality to this prototype biosensor by bolting on addition capabilities to measure other target analytes in strawberry fruit and other fresh produce types to further improve routine QC (10). Moreover, the storage stability of the GOx-based biosensor will be studied in future experiments in order to determine the suitability of the sensor for commercial applications.

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