AMPEROMETRIC FRUCTOSE SENSOR BASED ON FERROCYANIDE MODIFIED SCREEN-PRINTED CARBON ELECTRODE

Julien Biscay[a], Estefanía Costa Rama[a], María Begoña González García[a], A. Julio Reviejo[b], José Manuel Pingarrón Carrazón[b], Agustín Costa García[a]*

[a]Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo, 33006 Oviedo, Spain

[b]Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, 28040 Madrid, Spain

**e-mail: costa@uniovi.es*

Abstract: The first fructose sensor using a commercial Screen-Printed Ferrocyanide/Carbon Electrodes (SPFCE) is here reported. The ferrocyanide is included in the carbon ink of the commercial screen-printed carbon electrode. The immobilization of enzyme D-fructose dehydrogenase (FDH) was carried out in an easy way. An aliquot of 10 μL FDH was deposited on the electrode surface and left there until dried (approximately 1 h) at room temperature. The sensor, so constructed, shows a good sensitivity to fructose (1.25 μA/mM) with a slope deviation of ± 0.02 μA/mM and a linear range comprised between 0.1 and 1 mM of fructose, with a limit of detection of 0.05 mM. These sensors show good intersensors reproducibility after a previous pretreatment and a high stability. Fructose was determined in real samples as honey, Cola, fruit juices (orange, tomato, apple and pineapple), red wine, red and white grapes, musts and liquor of peach with a good accuracy.

Keywords: ferrocyanide, fructose dehydrogenase, fructose sensor, Screen-Printed Carbon Electrode

1. Introduction

Determination of sugars in food **[1]** and biological fluids **[2, 3]** for quality control and disease diagnostics is of paramount importance. D-Fructose, one of the principal sugar components, is a widely distributed monosaccharide and an important sweetener. Several analytical methods for the determination of D-fructose such as fluorometric [**4, 5**], gas chromatography [**6**], liquid chromatography [**7**], Fourier transform mid [**8**] and near infrared spectroscopy [**9**], coulometric [**10**], electrochemistry [**11**] have been described in the literature. These methods often are expensive, time consuming and require elaborate sample pretreatment [**7**]. Enzyme kits are also available for fructose determination using a couple-enzyme system. The advantage of the enzymatic determination relies on the inherent selectivity of enzymes and the short analysis time. The enzyme D-fructose dehydrogenase was isolated and characterized for the first time by Yamada and al. who confirmed that the enzyme catalyzes the oxidation of D-fructose to 5-keto-fructose in the presence of mediator **[12]** as for example, ferrocene [**13**], Meldola Blue [**14**], and ferricyanide [**15**]. FDH is an enzyme containing pyrroloquinolinequinone (PQQ) and belongs to a group of quinoproteins that have been described as a good alternative for the construction of enzymes electrodes [**16**]. The stability and the biological function of the enzyme depend of the immobilization of the mediator and the enzyme. Thus, different enzyme immobilization techniques as

adsorption [**17**], cross-linking [**18, 19**], entrapment [**20, 21**] or electropolymerization [**22**] are carried out in order to ensure the stability of the sensor.

Considering the disadvantages of the classical methods, the development of a portable, rapid, accurate and reproducible sensor is of a great interest. In the literature, few articles about fructose sensor using the screen printing technique have been found [**23-24**]. The pretreatment and the modification of the electrodes are more complicated and longer. In those cases, the screen printed electrode was fabricated in the laboratory and ferricyanide or phenazine methansulfate were used as mediators. The present work describes the design of the first fructose sensor using a commercial Screen-Printed Ferrocyanide/Carbon Electrode (SPFCE). The sensor developed in this work was obtained by the simple adsorption of FDH on the SPFCE surface. Experimental parameters, as applied potential, pH of the buffer solution and the concentration of the enzyme have been optimized. Analytical performances, in terms of reproducibility, limit of detection, linear range, stability and viability to measure in real sample have been reported too and are acceptable in comparison with the others fructose sensors based on SPE in particular.

2. Experimental

2.1 Chemicals

D-Fructose Dehydrogenase from *Gluconobacter industrius,* (FDH; ref. F4892), D-(-)-Fructose (F0127), fructose assay kit (ref. FA-20) and glucose assay kit (ref. GAGO-20) were purchased from SIGMA (Madrid, Spain). Potassium chloride (ref.596470), sodium hydroxide, sulfuric acid (ref. 1.00731.1011) and copper sulfate (ref.102780) were delivered by Merck (Spain). All chemicals were of analytical reagent grade, and the Milli-Q water used was obtained from a Millipore Direct-Q^{TM} 5 purification system.

Stock solutions of fructose and FDH were prepared daily in 0.1 M Phosphate Buffer Solution (PBS) of pH 4.5 for an immediate use. Britton Robinson buffer solutions of pH values 3 and 9 were used for pH studies.

2.2 Apparatus and Measurements

Chronoamperometric measurements were performed using an ECO Chemie μAutolab type II potentiostat interfaced with a Pentium 166 computer system and controlled by the Autolab GPES software version 4.8 for Windows 98. All measurements were carried out at room temperature. Screen-Printed Ferrocyanide/Carbon Electrodes (ref DRP-F10) and an edge connector (ref. DRP-DSC) were purchased from DropSens, S.L (Oviedo, Spain). These sensors consist in a Ferrocyanide/Carbon working (4 mm diameter), carbon auxiliary and silver pseudo reference electrodes printed on an alumina substrate. An insulating layer serves to delimit the electrochemical cell and electric contacts. Spectrophotometric measurements were performed using a spectrophotometer SPECTRONIC 20 GENESIS.

2.3 Electrode modification

After a first step of washing, 40 μL of the buffer (0.1 M PBS, pH 4.5) was deposited on the SPFCE and a potential of + 0.25V was applied to reach an intensity of 1.8 μA. Then, an aliquot of 10 μL of FDH (0.125 U/μL) was put onto the electrode surface and leaving there until dryness (1h). After a second washing step, the sensor can be used or kept into a freezer at – 20 °C and protected from light.

2.4 Analytical signal recording

To obtain the analytical signal, an aliquot of 40 μL of fructose solution was deposited on the sensor. The chronoamperogram was recorded applying a potential of + 0.25 V during 100 s. A different sensor was used for each measurement.

2.5 Real Sample Measurement

The sensor developed in this work was tested in different real samples (red wine, musts, honey, Cola, orange juice, pineapple juice, tomato juice, and apple juice). 1 g of honey was diluted in 50 mL of deionized water and diluted 100 times in the buffer solution. The fruit juices were diluted 200 times while the Cola, the musts have been diluted 2000 times, the red wine 100 times and the liquor of peach 1000 times. In the case of the grapes, a pretreatment was necessary, centrifugating them during 5 min at 5000 rpm. Then the supernatant was diluted 1000 times with the buffer. 40 μL of each sample were dropped on different sensors and the chronoamperogram was recorded as explained in the section 2.4. The obtained results with these food samples were compared with those obtained with two enzymatic spectrophotometric commercial kits. Samples were also prepared and tested following the instructions of the fructose and glucose enzymatic kits. Fructose kit is based on the phosphorylation of the D (-) fructose by adenosine trisphosphate to D (-) fructose 6-Phosphate with the formation of adenosine-5V-diphosphate (ADP). Fructose 6-phosphate is converted to glucose 6-phosphate by phosphoglucose isomerase (PGI) and this later is oxidized to 6-phosphogluconate in the presence of nicotinamide adenine dinucleotide (NAD) catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). The reduced form of NAD,

NADH, formed during the oxidation of D-glucose-6-phosphate is measured at 340 nm. Glucose kit is based on the spectrophotometric detection of the reaction product formed in the reaction between H_2O_2 and the reduced form of the o-dianisidine. Then the reaction of the sulfuric acid with the oxidized o-dianisidine formed a pink colored and more stable product. The intensity of the pink color is proportional to the original glucose concentration and measured at 540 nm.

3. Results and discussion

3.1 Optimization of parameters that affect the analytical signal

3.1.1 Study of the applied potential

First of all, the potential applied to detect fructose is a critical parameter due to two reasons: The potential applied must oxidize the ferrocyanide to ferricyanide which reacts with the fructose according to the following reaction:

$$\text{fructose} + \text{ferricyanide} \xrightarrow{\text{FDH}} \text{keto-fructose} + \text{ferrocyanide}$$

Moreover the potential applied must allow detecting the ferrocyanide enzymatically generated. This potential must be high enough to oxidize the ferrocyanide and in the same time allow discriminating the ferrocyanide enzymatically generated from the ferrocyanide electrochemically oxidized. The mechanism of the reaction is resumed in the **figure 1**.

After a first washing step, 10 µL of FDH (0.5 U/µL) were deposited and left to dry 1h. After a second washing step, the analytical signal was recorded according to the section 2.4 with 40 µl of 10^{-3} M of fructose, applying to each sensor a different potential (+ 0.1 to + 0.4 V). The results obtained are shown in the **Fig 2**.

The analytical signal increased with potential until + 0.3 V, but the background signals became more important at more positive values of potentials, because more ferrocyanide was oxidized to ferricyanide. For higher potentials, the response decreased and the background was more important. The best potentials for the measurement were between + 0.2 and + 0.3 V where the highest signal recorded and the lowest value of the background were obtained. For further experiments, the applied potential was + 0.25 V. For lower values of potential than + 0.2 V, the analytical response was lower due to the reduction of the ferricyanide to ferrocyanide. However, despite the response/background ratio obtained at chosen potential was (+ 0.25 V) high enough, the background signal was very high and gave rise a bad intersensor reproducibility. In order to decrease the background signal and improve the intersensor reproducibility, an electrochemical pretreatment was carried out. After a washing step, an aliquot of 40 µL of 0.1 M PBS (pH = 4.5) was deposited on the sensor and a chronoamperogramm was recorded applying + 0.25 V (during ca. 80 s) until to obtain a basal signal of 1.8 µA. In that way, all the electrodes were similar because part of ferrocyanide has been oxidized and removed of the electrode surface and consequently, lower backgrounds were obtained. The results of the pretreatment on the background and on the signal recorded are resumed on the **Table 1**.

Although the analytical signal obtained with pretreatment was lower the signal/background ratio was 7 fold times higher. In all cases of fructose sensor using SPE, a longer pretreatment was necessary (**Table 6**).

3.1.2 Optimization concentration of the enzyme

Different sensors were prepared, dropping different FDH concentrations. For each concentration of enzymes, a chronoamperogram with a fructose concentration of 10^{-3} M was recorded as described in section 2.4. The result of this study is reported in **Fig 3.**

It can be noted that the analytical signal increased when concentration of FDH increased. The background, after an initial increase, keep constant for a concentration of FDH comprise between 0.125 and 0.5 U/μL. It was chosen a concentration of FDH of 0.125 U/μL, because the analytical signal was considered quite high with excellent intersensors reproducibility and moreover cost of the sensor was lower.

3.1.3 Effect of the pH

The influence of the pH of the substrate was tested. Fructose solutions were prepared in Britton-Robinson solutions for the pHs 3 and 9. For the pHs between 3 and 9, fructose was prepared in 0.1 M PBS buffer. Sensors were prepared as explained in section 2.3 and the chronoamperometric signal was recorded as described in section 2.4. The results obtained are shown in the **Fig 4**. The analytical signal increased with pH until a pH value of 4 when a plateau was reached between 4 and 5. At higher pH values the sensor response decreased. Moreover, the background was smaller in the range of better response. To complete the study, glucose response was checked between pHs 3 and 9. So glucose solutions were prepared in Britton-Robinson solutions for the pHs 3 and 9. For the pHs between 3 and 9, glucose was prepared in 0.1 M PBS buffer. The results are resumed in the **Figure 4.** The response of the sensor to the presence of glucose increased between pHs 4 and 9. For further studies a pH of 4.5 was chosen, because it

corresponds to the optimal pH of the enzyme and to the soluble FDH with ferricyanide and in the same time the interference caused by the glucose is smaller **[25].**

3.2 Calibration of the sensor

Chronoamperograms corresponding to aliquots of 40 μL of different concentration of fructose were recorded to check the response of the electrodes in presence of fructose. **Fig 5A** shows the calibration curve obtained. The sensor shows Michaelis-Menten kinetics. Using the Lineweaver-Burk linearization, the Michaelis Menten constant (Km) was calculated and the value was 0.9 ± 0.1 mM. This value is lower than the value obtained with FDH immobilized on a multi walled carbon nanotubes modified Platinum electrode **[26]** and in the same range of sensors using a cellulose acetate membrane [**13**]**.** The linear range is displayed in the **Fig 5B**. A linear relationship between current and FDH concentration in the range of 0.1 and 1 mM was obtained with a coefficient of determination of 0.998 according to the following equation:

$$i_c = 1.27\ C_{fructose} + 0.15$$

The present sensor shows a linear range similar or better than the other sensor in the same category **(Table 5)**.

In order to evaluate the reproducibility of the sensor, series of 18 electrodes were prepared and tested the same day. This operation was repeated on three different days. A calibration plot of each series was carried out with solutions of fructose prepared the day of the measurement. The results are shown in the **Table 2.** The sensor has a good reproducibility and a slope of 1.25 ± 0.02 μA/mM. This reproducibility allows the

detection of fructose with a simple measurement (one standard and the sample). The relative standard deviation of the different slopes and the sensitivity obtained (1.9% and 1.25 μA/mM) are excellent in comparison with the other fructose sensor based on screen printed electrode or fructose sensors in general **(Table 5)**.

3.3 Specificity of the sensor

The specificity of the sensor was checked under the experimental conditions explained in the section 2.4. The potential interferents tested were ascorbic acid and another sugar such as glucose. To evaluate these interferences, solutions of 5 x 10^{-4} M of glucose and fructose and another one of 2.5 x 10^{-5} M of ascorbic acid, mixture of fructose (5 x 10^{-4} M) and glucose (5 x 10^{-4} M) and another one of ascorbic acid (2.5 x 10^{-5} M) and fructose (5 x 10^{-4} M) were prepared. To eliminate interferences caused by the ascorbic acid, solutions containing this interfering agent were prepared and treated with copper sulfate. So mixture of ascorbic acid (2.5 x 10^{-5} M) and copper sulfate (2.5 x 10^{-5} M) and fructose (5 x 10^{-4} M) and another mixture of ascorbic acid (2.5 x 10^{-5} M) and copper sulfate (2.5 x 10^{-5} M) were prepared. 40 μL of those solutions were dropped on the sensor and chronoamperograms were recorded as explained in the section 2.4. The different results obtained are resumed in the **Table 3**. The present sensor shows a good specificity for the fructose. In presence of glucose and ascorbic acid no measurable amperometric response could be observed. The signals recorded for the glucose and ascorbic acid measurements are equal as the recorded for the background.

3.4 Stability of the sensor

Several fructose sensors were prepared as described in the section 2.3, kept into the refrigerator (4 °C) or a freezer (-20 °C) and light protected until their use. A calibration

plot was carried out in the range of 0.1 to 1 mM after one and two months. The calibration plots are summarized in the **Table 4.** When the sensor is kept in refrigerator, it can be observed after one month a decrease of 20% of the slope. On the other hand, when sensors are kept at -20 °C, the sensitivity decreased about 5 % after two months. This stability is very good compared with other works and the best regarding the other screen printed fructose sensors already published.

4. Application to real sample

The proposed fructose sensor was used to measure fructose in real samples as honey, some fruit juices (apple, pineapple, orange, and tomato), red wine, musts, liquor of peach and grapes. The samples were prepared as explained in the section 2.5 and the analytical signal was recorded as described in the section 2.4. In all of the tested samples, the reference value indicated the amount of the addition of fructose and glucose. Recently, we have reported the construction of an amperometric sensor for glucose in which a mixture of glucose oxidase (Gox) and Horseradish peroxydase (HRP) were immobilized by adsorption on a SPFCE [**27**]. The results were compared by a volumetric method using ferricyanide for the qualitative determination of reducing sugars **[28]** and with two enzymatic commercial kits. In the case of the cola, pineapple and orange juice, the volumetric method could not be used. In that samples it has been used the value given on the bottle as reference. So fructose and glucose were determined in the samples exposed above and the results obtained were summarized in the **Table 6.** In all the cases, the proposed sensor shows a good accuracy with the results obtained with the reference and with the kits in a large range of sugars concentration. Seeing those results, it could be studied the eventuality of the construction of a very simple biosensor for the simultaneous detection of fructose and glucose by the

immobilization of a mixture glucose oxidase and horseradish peroxydase, and fructose dehydrogenase onto the surface of a Ferrocyanide/Carbon Screen Printed Electrode.

5. Conclusion

In this work, the first fructose sensor based on a commercial screen printed electrode is reported. This single-use fructose sensor can operate under air by immobilizing FDH on a Screen-Printed Ferrocyanide/Carbon Electrode. The biosensor transducer is a commercial screen-printed electrode which has the mediator included in the working electrode; this SPFCE needs a pretreatment to be used as transducer. Moreover, the biosensor is very easily obtained by simple adsorption of the enzyme onto the working electrode with no need of cross-linking agents or polymers. The resulting sensor displays low detection limits, high reproducibility, long term stability for fructose determination and linear response range from 0.1 to 1 mM with sensitivity of 1.25 ± 0.02 μA/mM. Furthermore the sensor can analyze fructose in sample containing glucose without its elimination and with a minimum sample preparation. Finally, interferences provocated by the presence of the ascorbic acid was not a problem with the studied samples.

Acknowledgements

This work has been supported by Spanish Ministry of Science and Innovation Project PET 2008_0174_02

References

[1] P. A. Paredes, J. Parellada, V.M. Fernández, I. Katakis, E. Domínguez, Biosens and Bioelectronics 12 (1997) 1233-1243.

[2] A. Lindqvist , A. Baelemans, C. Erlanson-Albertsson, Regulatory Peptides 150 (2008) 26–32.

[3] K. Mellor, R. H. Ritchie, G. Meredith, O. L. Woodman, Margaret J. Morris, L. M. D. Delbridge, Nutrition 26 (2010) 842–848.

[4] N. D. Danielson, C. A. Heenan, F. Haddadian, A. Numan, Microchem. Journal 63 (1999) 405-414.

[5] W. Tan, D. Zhang, Z. Wang, C. Liu, D. Zhu, J. Mater. Chem.17 (2007) 1964–1968.

[6] P. N. Wahjudi , M. E. Patterson , S. Lim , J. K. Yee , C. S. Mao, W.-N. P. Lee, Clinical Biochem, 43(2010) 198-207

[7] J. H. Han, H. N. Choi, S. Park, T. D. Chung,W-Y. Lee, Anal. Sciences, 26 (2010) 995-1000.

[8] S. Bureau, D. Ruiz, M. Reich, B. Gouble, D. Bertrand,J-M. Audergon, C.M.G.C. Renard, Food. Chem 115 (2009) 1133-1140.

[9] L. Xie, X. Ye, D. Liu, Y. Ying, Food Chem 114 (2009) 1135–1140.

[10] S. Tsujimura, A. Nishina, Y. Kamitaka, K. Kano, Anal. Chem. 81 (2009) 9383–9387.

[11] S. Campuzano, V. Escamilla-Gómez, M.A. Herranz, M. Pedreroa, J. M. Pingarrón, Sensors and Actuators B 134 (2008) 974–980.

[12] Y.Yamada, K. Aida, T. Uemura, Agric. Biol. Chem, 30 (1996) 95-96.

[13] J. Tká, I. Voštiar , E. Šturdík, P. Gemeiner ,Vladimír Mastihuba , J. Annus, Anal. Chim. Acta 439 (2001) 39–46.

[14] C. A. B. García, G. de Oliveira Neto, L. T. Kubota, L. A. Grandin, J. Electroanal. Chem. 418 (1996) 147-151.

[15] C. A. B. García, G. De Oliveira Neto, L.T. Kubota, Anal. Chim. Acta. 374 (1998) 201-208.

[16] M. Smolander, G. M. Varga, L. Gorton, Anal.Chim.Acta 302 (1995) 233-240.

[17] A. Kusakari, M. Izumi, H. Ohnuki, Colloids and Surfaces A: Physicochem. Eng. Aspects 321 (2008) 47–51.

[18] R.Rajkumar, A. Warsinke, H. Möhwald, Frieder W. Scheller, Martin Katterle, Talanta 76 (2008) 1119-1123.

[19] S. Campuzano, O. A. Loaiza, M.Pedrero, F. J. M. de Villena, J. M. Pingarrón, Bioelectrochemistry 63 (2004) 199-206.

[20] S. M. Reddy, P. Vadgama, Anal. Chim. Acta 461 (2002) 57-64.

[21] R. Ben-Knaz, D.Avnir, Biomaterials 30 (2009) 1263-1267.

[22] A. S. Bassi, E. Lee, J. X. Zhu, Food Research International 31 (1998) 119-127.

[23] U.B. Trivedi, D. Lakshminarayana, I. L. Kothari, P. B Patel, C. J. Panchal, Sens. Actuat. B 136 (2009) 45-51.

[24] S. Piermarini, G. Volpe, M. Esti, M. Simonetti, G. Palleschi, Food Chem 127 (2011)749-754.

[25] M. Ameyama, E. Shinagawa, K. Matsushima, O. Adachi, J. Bacteriol. 145 (1981) 814-823.

[26] M. Tominaga, S. Nomura, I. Taniguchi, Biosens and Bioelec. 24 (2009) 1184-1188.

[27] J. Biscay, E. Costa Rama, M. B. González García, J.M. Pingarrón Carrazón, A.Costa García, Electroanal 23 (2011) 209-214.

[28] S.W. Cole, Biochem J. 27-3 (1933) 723-726.

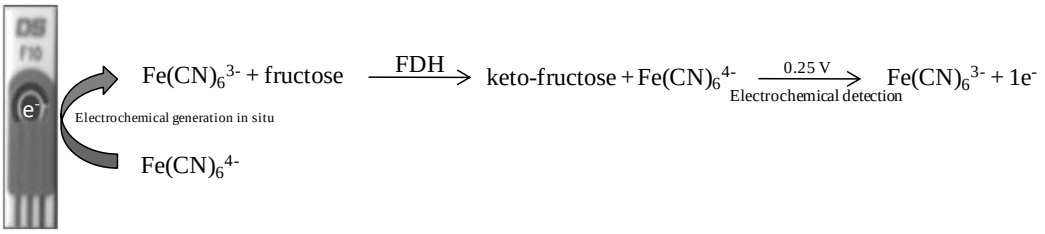

Figure 1. Enzymatic reaction at the electrode surface.

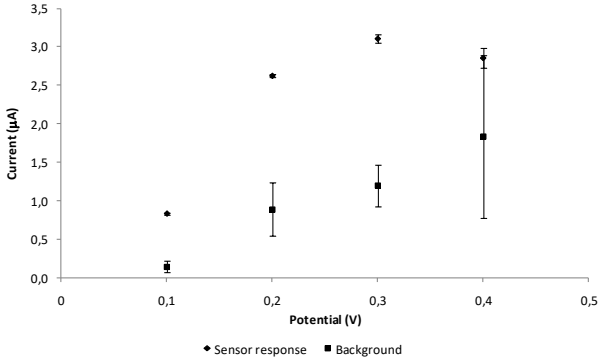

Figure 2. Effect of the applied potential on the analytical signal. $C_{fructose} = 10^{-3}$ M in 0.1 M PBS (pH=6); C_{FDH} = 0.5 U/µL in 0.1 M PBS (pH=4.5). $t_{recording}$ = 100 s, Data are given as average ± SD (n=3).

	Without pretreatment		With pretreatment	
	Current (μA)	**RSD %**	**Current (μA)**	**RSD %**
Background	0.97	45	0.05	20
Signal	3.7	36	1.4	8
Signal/background	3.8		28.0	

Table 1. Effect of pretreatment on the analytical and background signal. Fructose concentration, 10^{-3} M; C_{FDH} = 0.125 U/μL in 0.1 M PBS (pH=4.5), $E_{applied}$= + 0.25 V (vs Ag pseudo reference electrode), $t_{recording}$ = 100 s. Data are given as average (μA), each point was measured three times**.**

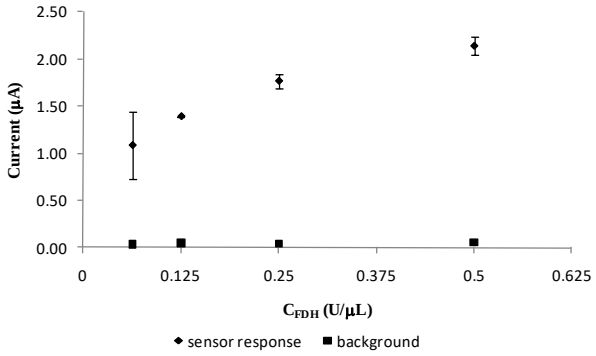

Figure 3. Effect of the concentration of enzyme FDH on the analytical signal and on the background. $C_{fructose}$ = 10^{-3} M in 0.1 M PBS (pH= 4.5); $E_{applied}$= + 0.25 V (vs Ag pseudo reference electrode), $t_{recording}$ = 100 s. Data are given as average ± SD (n=3),

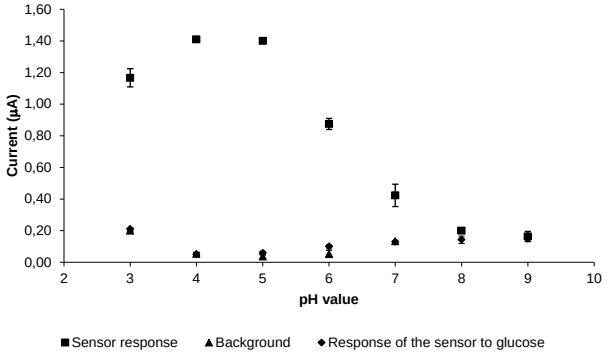

Figure 4. Effect of the pH value of the substrate and glucose on the response of sensors to 10^{-3} M fructose and 10^{-3} M glucose, C_{FDH} = 0.125 U/µL; $E_{applied}$= + 0.25V (vs Ag pseudo reference electrode), $t_{recording}$ = 100 s, each point was measured three times.

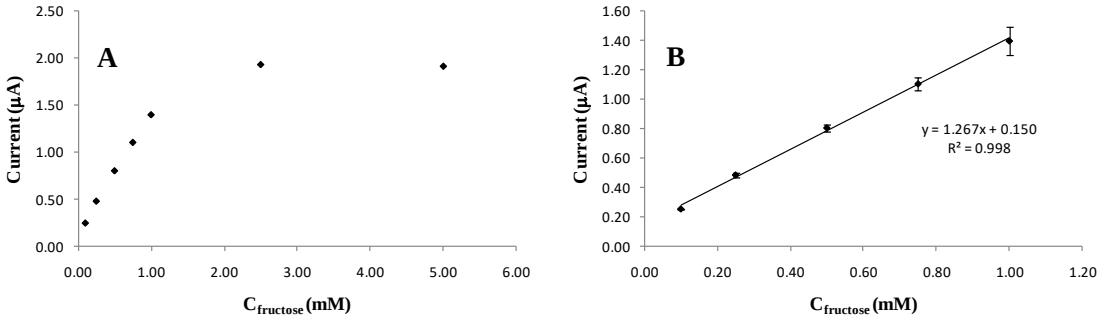

Figure 5. Calibration curve and calibration plot of the proposed fructose sensor in the concentration range 10^{-4}-5 x 10^{-3} M. $C_{fructose}$ = 10^{-3} M in 0.1 M PBS (pH=4.5), $E_{applied}$= + 0.25V (vs Ag pseudo reference electrode), $t_{recording}$ = 100 s Data are given as average ± SD (n=3).

	Equation	R^2
Calibration plot 1	$i(\mu A) = 1.27\ C_{fructose} + 0.15$	0.998
Calibration plot 2	$i(\mu A) = 1.23\ C_{fructose} + 0.16$	0.995
Calibration plot 3	$i(\mu A) = 1.26\ C_{fructose} + 0.17$	0.992
Calibration plot 4	$i(\mu A) = 1.22\ C_{fructose} + 0.18$	0.99
Mean slope	$1.25 \pm 0.02\ \mu A/mM$	

Table 2. Calibration plot equations of three fructose sensor series, C_{FDH} = 0.125U/μL in 0.1 M PBS (pH=4.5), $E_{applied}$= + 0.25V (vs Ag pseudo reference electrode), $t_{recording}$ = 100 s, n=6 in all calibration plots; each point was measured three times.

background	fructose	glucose	fructose/glucose	pretreated ascorbic acid	fructose/pretreated ascorbic acid
(45 ± 7) nA	(810 ± 20) nA	(47 ± 6) nA	(825 ± 50) nA	(50 ± 5) nA	(810 ± 30) nA

Table 3. Study of the interferences caused by glucose and ascorbic acid. C_{FDH} = 0.125U/µL, $C_{glucose} = 5*10^{-4}$ M, $C_{ascorbic\ acid} = 2.5 \times 10^{-5}$ M, $C_{fructose} = 5 \times 10^{-4}$ M, all the dissolutions are prepared in 0.1 M PBS (pH=4.5), $E_{applied}$= + 0.25V (vs Ag pseudo reference electrode), $t_{recording}$ = 100 s, each point was measured three times. data are given as average ± SD (n=3)

	Freezer		Refrigerator	
	Equation	**R^2**	**Equation**	**R^2**
Calibration plot (1 day)	$i\ (\mu A) = 1.25\ C_{fructose} + 0.17$	0.994	$i\ (\mu A) = 1.25\ C_{fructose} + 0.17$	0.994
Calibration plot (1 month)	$i\ (\mu A) = 1.18\ C_{fructose} + 0.14$	0.996	$i\ (\mu A) = 0.99\ C_{fructose} - 0.05$	0.999
Calibration plot (2 months)	$i\ (\mu A) = 1.18\ C_{fructose} + 0.17$	0.995	—	—

Table 4. Calibration plot of electrodes stored during different times. n=6 in all calibration plot. C_{FDH} = 0.125 U/μL or FDH/BSA (0.125 U/μL; 0.1 % respectively) in 0.1 M PBS (pH 4.5), $E_{applied}$= + 0.25 V (vs Ag pseudo reference electrode), $t_{recording}$ = 100 s, each point was measured three times.

Electrode modification	Mediator	Type of electrode	Pretreatment	Potential (V)	Sensitivity	Detection limit (mM)	Linear range	K_m (mM)	Reproducibility (RSD %)	Stability	Reference
Adsorption of FDH	Ferrocyanide	SPFCE	Applying + 0.25 V to reach 1.8 mA (80 s)	+ 0.25	1.25 $mA.mM^{-1}$	0.05	(0.1-1) mM	0.9	1.9	No lost of sensitivity after 60 days at -20 ° C	Present work
Entrapment of FDH in a polymer matrix	Ferricyanide	SPE	Applying + 1.2 V during 240 s	+ 0.4	0.62 $mA.mM^{-1}$	0.65	(3-13) mM	not reported	not reported	more than 50 % of sensitivity lost after 30 days	[23]
Adsorption of FDH/BSA/glutaraldehyde mixture	Phenazine methansulphate	Bare graphite SPE	Applying + 1.7 V during 180 s	+ 0.07	not reported	not reported	(0.05-0.5) mM	not reported	7	10 % of sensitivity lost after 15 days	[24]
Adsorption of FDH	none	MWCNT modified Platinum electrode	none	- 0.15	not reported	5	up to 40 mM	11	not reported	3 days	[26]
FDH coated on ferrocene-embedded cellulose acetate membrane	Ferrocene	Glassy Carbon Electrode	none	+ 0.300	20 $nA.mM^{-1}$	7	not reported	not reported	not reported	9 h	[13]
FDH coated with Meldola Blue onto a silica gel	Meldola Blue	Silica gel modified carbon electrode	none	+ 0.02	0.618 $mA.mM^{-1}.cm^{-2}$	not reported	(0.1-0.8) mM	not reported	0.68	2 months	[14]
Cross linking with glutaraldehyde	Tetrathiafulvalene	Gold electrode	Polishment, sonification, immersion in KOH, H_2SO_4, and HNO_3	+ 0.2	1.7 $mA.mM^{-1}$	0.002	(0.01-1)mM	5.4	not reported	30 days	[29]

Table 5. Analytical characteristics of some fructose sensors.

Real Sample	fructose sensor	fructose sensor (kit)	glucose sensor	glucose sensor (kit)	Σ glucose and fructose (sensor)	Σ glucose and fructose (kit)	Reference (Volumetric method)
Honey (g/100 g)	29.1 ± 0.4	31	34 ± 1	35.00	63 ± 1	66	69
Tomato juice (g/100 mL)	1.7 ± 0.2	1.5	1.10 ± 0.01	1.00	2.8 ± 0.2	2.5	2.80
Pineapple juice*(g/100 mL)	3.22 ± 0.08	3.3	1.50 ± 0.03	1.60	4.72 ± 0.09	4.9	5.20
Orange juice*(g/100 mL)	3.4 ± 0.2	3.4	1.60 ± 0.05	1.50	5 ± 0.2	4.9	5.20
Red wine (g/L)	5.3 ± 0.1	5.5	2.3 ± 0.1	2.40	7.6 ± 0.2	7.9	7.7 ± 0.6
Apple juice (g/100 mL)	7.68 ± 0.01	7.4	2.7 ± 0.2	2.80	10.4 ± 0.1	10.2	11.85
Coca cola*(g/100 mL)	8.0 ± 0.1	8.3	2.80 ± 0.05	3.00	10.8 ± 0.1	11.3	10.60
Red must (g/L)	41 ± 1	40	73 ± 1	73.00	114 ± 1	113	130 ± 8
White must (g/L)	43 ± 1	42	84 ± 1	81.00	127 ± 1	123	126 ± 13
Red grapes (g/L)	62 ± 4	63	89 ± 2	92.00	151 ± 5	155	147 ± 10
White grapes (g/L)	53 ± 2	55	116 ± 2	113.00	169 ± 3	168	160 ± 7
Liquor of peach (g/L)	74 ± 2	80	170 ± 10	183.00	244 ± 9	263	283 ± 9

Table 6. Measurement of fructose and glucose with the proposed sensor and a previously published glucose sensor, in real samples. C_{FDH} = 0.125U/μL in 0.1 M PBS (pH=4.5), $E_{applied}$= + 0.25 V (vs Ag pseudo reference electrode), $t_{recording}$ = 100 s; Data are given as average ± SD (n=3).