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Enzyme from Banana (*Musa* sp.) Extraction Procedures for Sensitive Adrenaline Biosensor Construction

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ABSTRACT

For building a biosensor, at least two enzyme sources can be employed. The pure enzyme has features with better selectivity and low stability. Crude extract presents better stability and wrong selectivity. Thus, one intermediate condition can be feasible joining both benefits of crude extract and pure enzymes. For that result, several procedures of extraction and semi purification of polyphenol oxidase (PPO) enzyme from banana (*Musa* sp.) were studied. The results showed that cleaned enzymatic extracts presented higher specific activities than crude extracts (2.8 up to 5.3 fold), despite the total protein concentration diminishing from 27% up to 72%, indicating that polyphenol oxidase (PPO) enzyme was preserved in them. The biosensors with 125 AU mL^{-1} containing cleaned enzymatic extracts performed better by grinding or grinding plus sonication for 30 s. They were linear over the ranges of 5.9×10^{-6} mol·L^{-1} to 1.4×10^{-3} mol·L^{-1} and 7.9×10^{-8} mol·L^{-1} to 4.0×10^{-3} mol·L^{-1}, respectively. The limit of Detection (LOD) was 5.9×10^{-6} mol·L^{-1} and 7.9×10^{-8} mol·L^{-1}, respectively. The LOD obtained is adequate to adrenaline determination on blood and medicinal samples, and were applied in medicinal samples with satisfactory results.

Keywords: Enzymatic Extract; Epinephrine; Biosensor; Polyphenol Oxidase

1. Introduction

Biosensor, which is built by using enzymes as biological components, is a very promising branch of analytical chemistry with several daily applications [1]. However, the use of enzymes faces the following dilemma: the pure enzyme has a better selectivity, but with low stability and high cost; on the other hand, the enzymatic crude extract has low cost and poor selectivity, but higher stability. Considering this dilemma, one way to obtain better biosensor performance is to study enzyme extraction procedures searching one intermediate condition between pure and crude extract, combining the better features.

In the case of enzyme extraction, one should consider that this step must be the most appropriate for the analysis [2]. The procedures most commonly employed for biosensor preparation are those that use plant tissues. In this case, the tissues, which are cut into thin slices, are directly fixed on an electrode [3-7]. These procedures present some problems, such as reproducibility of the biosensor construction, and variability of enzyme concentration in plant tissue [7].

A good alternative is the use of powdered plant tissue, or its crude extract [7]. Using crude extract, rather than an isolated enzyme, represents an alternative with attractive analytical properties [1]. However, it is necessary to evaluate alternatives for the enzymatic extraction, since there are various procedures, both physical and chemical.

The tyrosinase enzyme is one of the most used enzyme in biosensor construction [8]. The tyrosinase (EC: 1.14.18.1) is also known as polyphenol oxidase (PPO), catechol oxidase and cresolase. This enzyme catalyzes the oxidation of both monophenols (e.g. tyrosine, phenol, p-cresol) and diphenols (e.g. catechol, L-dopa, dopamine, adrenaline) [9-11] leading to o-quinone, which then can polymerize and form melanin [12]. The PPO is widely distributed in nature, and found in various fruit and vegetable tissues [6,13]. This enzyme is responsible for the darkening of many fruits and vegetables when cut and exposed to air. In plants, the PPO is located in chloroplasts, and its concentration depends on the planting location, time of harvest, species and maturation stage [14].

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In the present work, the plant chosen as source for the enzyme PPO was the banana (Musa sp.), due to its abundance, high enzyme activity and ability to control the harvest time [15].

The studied analyte in this work was the adrenaline, which reacts with oxygen in the presence of PPO. The catecholamines are released by the sympathetic nervous system and adrenal medulla, to the circulating blood. About 80% of the catecholamines released by the adrenal medulla corresponding to adrenaline, acting in the heart, blood vessels, blood pressure, metabolism, central nervous system and smooth muscles [16].

The biosensor is one of the methods used for adrenaline determination, which has good stability, low construction and storage cost, potential for miniaturization, automation simplicity, and construction portability [7,17, 18].

Amperometry, spectrophotometry and potentiometry have been employed as transducers [12]. The potentiometric transducer is very common in analytical laboratories, due to its use in a simple pH-meter. Thus, its utilization for biosensors is feasible.

In previous work, good results, in terms of detection limits, linear range and sensibility, were achieved with a simple potentiometric transducer [12].

This study's objective was to investigate PPO extraction procedures from banana tissue to improve biosensor performance. We tried to obtain an enzymatic extract, where components which interfere with biosensor response would be removed, being maintained those that favor the keeping of its stability and selectivity. The various extracts were tested empirically to build a PPO carbon paste biosensor.

2. Experiment

2.1. Reagents and Solutions

The following reagents were used: Adrenaline, Sigma®; Dihydrogenphosphate, Synth®; Sodium Hydroxide, Vetec®; Polyvinylpyrrolidone (PVP), Synth®; Bovine Serum Albumin, Sigma®; Ethanol, Vetec®; Acetone, lsofar®; Graphite powdered, Synth®; Vaseline, Rioquimica®; Hydrogen Peroxide, Vetec®; Agar, Fluka®; Potassium Chloride, Sigma®; Dopamine, Fluka®; Urea, Vetec®; Noradrenaline, Fluka®; Uric Acid, Vetec®; Ascorbic Acid, Vetec®.

There was a study of interferent commonly found in blood samples, using adrenaline solutions 1.0×10^{-3} $mol \cdot L^{-1}$ in phosphate buffer 0.1 $mol \cdot L^{-1}$ (pH = 7.0). Three different concentrations for each of them were used. These values were found in the blood [19,20], and the interference test for lidocaine, present in anesthetic (Lidocaine 2%). The following compounds were used: ascorbic acid (0.100, 0.600 and 1.70 mg/100 mL), noradrenaline (0.850, 1.70 and 3.40 $mg \cdot L^{-1}$), urea (14.0, 40.0 and 80.0 $mg \cdot L^{-1}$), uric acid (50.0, 70.0 and 140 $mg \cdot L^{-1}$) and dopamine (15.0, 30.0 and 60.0 $ng \cdot L^{-1}$). All solutions were prepared with deionized water.

2.2. Apparatus

For preparing the enzyme extract, a chosen amount of peeled banana was weighed on an analytical balance (Sartorius®, model BP 210S, BR), to which phosphate buffer was added and ground in a blender (ARNO®, BR). Next, the enzyme extract suffered several sample preparation procedures as described in the section below, among them: sonication, where test tubes (in triplicate), containing the crude extracts, were placed in sonication bath (Quimis®, model Q335D, BR) and extraction by microwave irradiation, using a microwave oven (Milestone®, model Ethos plus, USA). The crude enzyme extracts were subjected to centrifugation (Fanem®, model Excelsa Baby I, BR).

Measurements for the determination of total protein and enzymatic activity were performed using an UV-Vis spectrophotometer (Shimadzu®, model UV-2400 PC, Japan). The pH meter (Schott®, model Handylab, GmbH) for preparation of buffers and water purification system (Millipore®, model Academic Milli-Q, USA) were used.

The potentiometric measurements were performed using a 12-bit interface (National Instruments, USA). For acquisition of data, the software was written in Visual Basic® language. The adrenaline and medicines determination was performed using a homemade autosampler [21], and the Ag/AgCl reference electrode was constructed according to Li *et al*. [22].

2.3. Procedures for Extraction of the PPO Enzymes

Ten enzymatic extraction procedures that included grinding, manual maceration, sonication and microwave radiation, as described in **Table 1**, always performed in triplicate, were proposed. 25 g of banana (*Musa* sp.) were used for each extract, added to 100 mL of dihydrogenphosphate buffer 0.1 $mol \cdot L^{-1}$ (pH = 7.00) and 2.5 g of polyvinylpyrrolidone (PVP) [12].

The first procedure studied was grinding in a blender, with a 3 min duration at room temperature, which has been one of the techniques employed in enzyme extraction from plant materials [3,7,17,23].

Manual maceration (procedure 2) consisted of a simple procedure that, depending on the sample physical properties, will assist in the plant's cell walls and organelles disruption, allowing more contact with the enzyme extraction solution. In this procedure the sample was placed in a porcelain mortar and then macerated at room temperature for 15 min [24].

Table 1. Procedures for extraction of enzymes.

Procedure	Description
1	Grinding in a blender for 3 min;
2	Manual maceration at room temperature for 15 min;
3	Grinding in a blender for 3 min, followed by manual maceration at room temperature for 15 min;
4	Grinding in a blender for 3 min, followed by sonication for 30 s;
5	Grinding in a blender for 3 min, followed by sonication for 60 s;
6	Grinding in a blender for 3 min, followed by sonication for 120 s;
7	Grinding in a blender for 3 min, followed by sonication for 180 s;
8	Grinding in a blender for 3 min, followed by extraction assisted by microwave, temperature of 37˚C for 1 min;
9	Grinding in a blender for 3 min, followed by extraction assisted by microwave, temperature of 37˚C for 3 min;
10	Grinding in a blender for 3 min, followed by extraction assisted by microwave, temperature of 37˚C for 5 min;

To investigate possible synergistic effects of the two procedures described above, the procedure 3 (**Table 1**) was suggested, which consisted in grinding followed by manual maceration.

Sonication for enzyme extraction has been proposed in this work. As an example, Becerra *et al.* [25] have used this procedure to extract intracellular yeast proteins, and found that sonication promoted a 50 times increase in protein extraction, when compared with other mechanical extraction procedures such as shaking by vortex or using glass beads.

Before applying the sonication to the ground enzyme extract, we performed a calibration of the ultrasonic bath, optimizing the test tube position into the bath unit [26]. The sonication time has significant influence on extraction efficiency. Therefore, 30, 60, 120 and 180 s of sonication were applied to assist the enzyme extraction after grinding the samples in a blender (procedures 4 to 7, **Table 1**).

The application of microwave radiation for the amino acid hydrolysis has become increasingly usual [27,28]. The main advantage is highlighted by several authors as to decreasing the hydrolysis time from hours to minutes. On the other hand, this radiation has also been used for proteolysis, such as enzyme extraction from human hair samples [29]. Zheng *et al.* [30] used microwaves at high temperatures (around 100˚C and 2450 MHz) for 5 min to rupture the wall cell for lipid extraction. With this procedure it was possible to increase lipid extraction 1.6 times, compared to the control. Based on these applications, the microwave radiation was used on procedures from 8 to 10, where 2 mL of extracts (obtained from the procedure 1) were submitted, in triplicate, to radiation at 37˚C temperature, with 1, 3 and 5 min periods of extraction time, respectively.

After finishing each procedure, the extracts were filtered through four fabric layers and centrifuged at 1800 x*g* for 30 min at room temperature. The supernatant solutions were stored in a refrigerator at 4˚C, and used subsequently for total protein concentration and enzyme activity determination [3,7,17,23].

2.4. Enzyme Activity and Total Protein Concentration Determination

The PPO activity present in plant crude enzymatic extract (CEE) was determined in triplicate, by measuring absorbance at 410 nm and monitoring the epinephrine-quinone formation [23].

We then measured the reaction rate for reaction mixtures containing 2.40 mL of adrenaline at a concentration of 5.0×10^{-2} mol·L^{-1} and CEE volumes of 0.05 to 0.20 mL. Phosphate buffer 0.1 mol·L^{-1} (pH = 7.00) to 3.00 mL was complete to volume in quartz cuvette. The enzyme activity is numerically equal to the speed reaction slope versus the enzyme amount multiplied by 1000 [15, 31].

The total protein concentration was determined by the Bradford's method [32,33]. This method is based on the interaction between the "Coomassie brilliant blue" BG-250 dye and the protein macromolecules, containing basic, or aromatic amino acid chains. At the reaction's pH, the interaction between the protein of high molecular weight and BG-250 dye causes shift in the dye equilibrium to the anionic form, which absorbs strongly at 595 nm [34]. The specific activity was also calculated, defined as the enzyme activity divided by total protein concentration, taking into account all dilutions in the preparation of enzyme extract.

The extracts that showed the highest values for PPO enzyme activity, for total protein concentration and stability, were selected for the next step of sample preparation, called "cleaning".

2.5. Enzyme Cleaning and Solubilization Step

Although some authors had argued that the enzymes in the crude extract are more stable, this being more advantageous for biosensor construction [7], we proposed a cleanup step targeting to eliminate possible interferences, such as salts, polysaccharides, phenols and possible damage to the biosensor performance. To this end, 1.00 mL of CEE was placed in a test tube (in triplicate) in which 3.00 mL of acetone/ethanol at 0˚C were added (in a 3:1 ratio (v/v)) in each tube, and taken to the freezer (–20˚C)

during 1 h for protein precipitation. Following that, the tubes were centrifuged at 1800 × g for 3 min, and the soluble part was removed. This cleaning procedure was repeated three times. After the acetone/ethanol mixture was completely evaporated (at room temperature) the proteins were solubilized with 1.00 mL of phosphate buffer 0.1 mol·L^{-1} (pH = 7.00).

2.6. Electrodes Construction

For biosensor construction, we used polyethylene tubes of 0.8 mm i.d.. The graphite paste was prepared with 0.375 g of graphite, 0.125 g of Vaseline and the enzymatic extract. A copper wire was used to make electrical contact [35]. It was constructed reference electrodes Ag/AgCl using polyethylene tubes 0.8 mm i.d. [35].

2.7. Potentiometric Measurement

A hydrogen peroxide (0.163 mol·L^{-1}) in phosphate buffer (pH = 7.0) solution was used as a blank. In a previous study it was found that hydrogen peroxide presence is necessary to avoid memory effects [12].

The signal records were performed for 10 min in triplicate [35], using the biosensor as working electrode and Ag/AgCl as reference electrode. For signal acquisition we used an interface National® 12-bit and a program written in Visual Basic® [21].

3. Results and Discussion

3.1. Enzyme Activity and Total Protein Concentration Determination

Table 2 shows the enzyme activity measurements and total protein concentration for the CEE with and without the cleaning step. This table also shows the specific activities, defined as enzyme activity divided by total protein concentration [35].

The second procedure (maceration) showed *ca.* 50% lower enzyme activity than the grinding in a blender (procedure 1). On the other hand, the grinding and macerating synergistic effect (procedure 3) enhances cellular organelle rupture, facilitating the enzyme extraction, which can be noted in the specific activity for the CEE.

The best sonification times were 30 and 60 s (procedures 4 and 5). The extracts submitted to the procedures 6 and 7, sonication longer than 60 s, showed enzymatic activity and total protein concentration comparatively lower than other procedures. The high powered ultrasonic waves cause permanent physical and chemical changes, because they produce cavitation and microfluidics in liquids [36]. It can be inferred that there was PPO enzyme denaturation to time exposures over 60 s.

Regarding the microwave radiation use, it may be noted that, under the experimental conditions used, these procedures (8 to 10) did not preserve the enzymes, since there was no significant enzyme activity.

In the cleaning step, there has been hydrosoluble and liposoluble enzyme separation. The former were precipitated, centrifuged and separated from the liposolubles, which were discarded along with the organic medium. Consequently, there was a decrease in total protein concentration, from 27% up to 72%, as observed for all enzyme extracts in **Table 2**. On the other hand, there was an increase in enzyme activity in cleaned extracts, compared to CEE. This fact shows that the PPO was preserved.

Phenolic compounds existing in crude extract are oxidized under PPO catalysis and this can inactivate enzymes [12]. In this context, these compounds separation, which are liposoluble during the cleanup step, confirms the increase in enzyme activity. Thus, it can be considered from **Table 2** that, under the conditions used in this work, and for this particular sample, the procedure involving grinding followed by maceration (procedure 3) and grinding followed by sonication for 30 or 60 s (procedures 4 and 5), with and without the cleanup step, were the most efficient for the extraction, and for the specific activity of PPO enzymes.

Table 2. Enzymatic activity, total protein concentration and specific activity of crude and cleaned enzymatic extracts.

Proc.	Type of enzyme extract	Enzymatic activity (AU·mL^{-1})	Total protein conc. (mg·mL^{-1})	Specific activity (AU mg of protein^{-1})
1	Crude	2558 ± 107	18.66 ± 0.67	137 ± 8
	Cleaned	3118 ± 114	9.75 ± 0.64	320 ± 24
2	Crude	1205 ± 46	10.23 ± 1.34	118 ± 16
	Cleaned	1359 ± 33	2.97 ± 0.27	458 ± 43
3	Crude	2379 ± 86	15.39 ± 0.68	155 ± 9
	Cleaned	3025 ± 113	6.87 ± 0.86	440 ± 58
4	Crude	2082 ± 93	16,83 ± 1,07	124 ± 10
	Cleaned	3166 ± 106	4.77 ± 0.85	664 ± 120
5	Crude	2253 ± 97	14.84 ± 1.35	152 ± 15
	Cleaned	2843 ± 86	6.51 ± 0.89	437 ± 61
6	Crude	273 ± 87	4.55 ± 0.95	60 ± 23
	Cleaned	343 ± 93	3.21 ± 0.89	107 ± 41
7	Crude	151 ± 77	3.44 ± 1.05	44 ± 26
	Cleaned	180 ± 81	2.51 ± 0.89	72 ± 41
8 to 10	Crude	nd*	< LOQ**	nd*

AU—Activity Unity; *nd = not detected; **LOQ = 6.71 × 10^{-5} mg·mL^{-1}.

3.2. Enzyme Extract Stability

Enzyme activity measurements and determination of total protein concentration were done for 18 days. The results are shown in **Figure 1**, in terms of specific activity.

The extracts obtained by procedures 1 and 3 showed little variation in the specific activity (11% and 8%, respectively): whereas the clean extracts had a decrease of specific activity until the eighteenth day (37% and 34%, respectively). In these cases the cleaning step contributed to the PPO inactivation more than for the total proteins concentration.

However, CEE extracted by procedures 4 and 5 had, respectively, 200% and 300% increase of specific activity. That showed that there was decrease in total protein concentration, with no PPO inactivation. No patterns were noted for these procedures with the cleaning step.

3.3. Enzyme Amount Influence on Biosensor

A study was conducted on the enzyme amount (activity unit, $AU \cdot mL^{-1}$) to be used in the biosensor construction. For this purpose, biosensors were constructed with 25, 50, 75, 100, 125 and 150 $AU \cdot mL^{-1}$ with enzymatic extract from procedure 1 (**Table 2**), and potentiometric measurements were made with adrenaline standard solution at a concentration of 1.0×10^{-4} $mol \cdot L^{-1}$. The biosensor constructed with 125 $AU \cdot mL^{-1}$ was the one with the largest analytical signal.

From these results, the cleaned extracts from extraction procedures 1 and 4 were chosen to build biosensors (called biosensors 1 and 2, respectively) and their analytical characteristics were evaluated.

3.4. Sensor Analytical Characteristics

Analytical parameter studies described in this section were made in accordance with IUPAC rules [37]. Biosensors 1 and 2 responses were linear over the 5.9×10^{-6} $mol \cdot L^{-1}$ to 1.4×10^{-3} $mol \cdot L^{-1}$ and 7.9×10^{-8} $mol \cdot L^{-1}$ to 4.0×10^{-3} $mol \cdot L^{-1}$ ranges, and the limits of Detection (LOD) were 5.9×10^{-6} $mol \cdot L^{-1}$ and 7.9×10^{-8} $mol \cdot L^{-1}$ (**Figure 2**), respectively. The obtained LOD's are adequate to adrenaline determination on blood and medicinal samples.

The biosensors were stable for a period of at least 30 days. During this interval, measurements were conducted under identical conditions using 1.0×10^{-3} $mol \cdot L^{-1}$ (pH = 7.0) epinephrine solution. Thus, one can study the drift parameter, which is the non-random electrode signal variation during a time span [38], keeping the same conditions in all measurements. We obtained the 0.94785 mV/day and 0.8175 mV/day slopes for biosensors 1 and 2, respectively.

The 18-day stability period for the enzyme extract is not presented as one of the best results ever reported in the literature. This is possibly due to the fact that the extracted enzyme group was separated from the original medium (crude extract). Periods of greater stability (up to 70 days) for CEE's have been reported in the literature [39]. However, the present paper authors believe that this is just one of several parameters to be evaluated and considered in the overall study context.

An interesting noticed aspect is the relationship between the results obtained for the LOD and stability. We observed an increasing sensitivity with cleaning endogenous interfering, concomitantly with decreased stability. This paper authors concept and in this context, considered that the better detectability is more desirable than long-term stability, since the raw material (banana) to build the biosensor is easily accessible and has low cost.

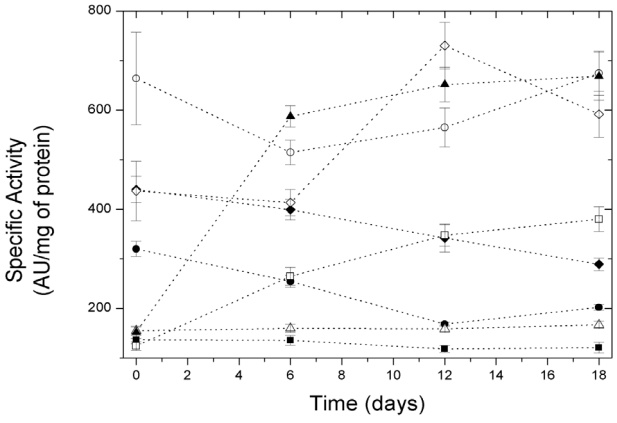

Figure 1. Determination of specific activity, in triplicates, during the period of eighteen days: (■) Procedure 1; (●) Procedure 1 with cleaning step; (△) Procedure 3; (◆) Procedure 3, with cleaning step; (□) Procedure 4; (○) Procedure 4, with cleaning step; (▲) Procedure 5; (◇) Procedure 5, with cleaning step.

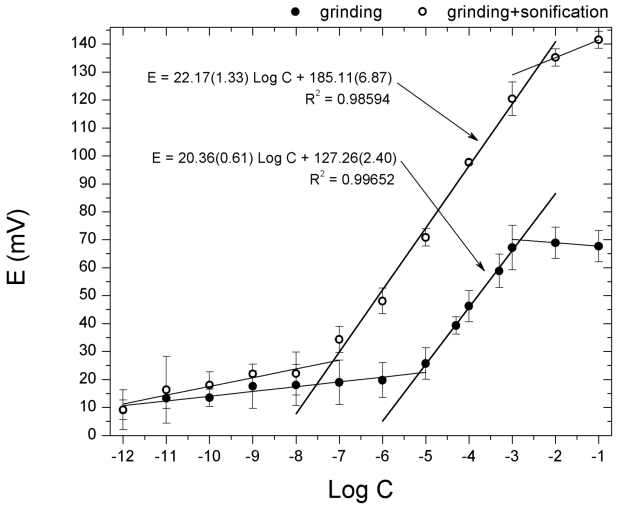

Figure 2. Analytical curves of biosensors 1, (●) grinding and 2, (○) grinding and sonication.

The precision [40] was calculated from the variation coefficient (CV), which was equal to 14.6% and 5.4% for biosensors 1 and 2.

The results for interference studies are presented in **Table 3**. There was no severe interference with both biosensors tested, when compared with other authors, as in the work developed by Mataveli *et al*. [12] and Abdel-Hami *et al*. [20], which showed interference problems, especially with ascorbic acid. Since comparing the studied biosensors 1 and 2, the second showed a greater selectivity for having a lesser influence from interfering in the analytical signal. These results are in agreement with the fact that when extracts are cleaned, their susceptibility to interference is decreased, because there is less enzyme variety to recognize the substrates, *i.e.*, only the analyte is detected. Thus, it is important to mention that this was this work's main objective, because biosensor selectivity was improved.

To check the accuracy [40], the performances of the proposed biosensors were compared with the spectrophotometric technique, described by the pharmacopoeia [41]. Two different epinephrine (commercial medicine) samples were used. The analyzed samples were an association of lidocaine hydrochloride 2% with adrenaline 1:100,000 and another drug sample containing 1.0 $g \cdot L^{-1}$ of epinephrine without concomitants. The results are shown in **Table 4**. The measurement precision by both methods was similar (checked with F-test) [42] and the accuracy was approved in the t-test with a 95% confidence level, showing that the methods are in agreement.

4. Conclusions

Regarding the enzyme extract, there was a better specific enzyme activity in cleaned protein extracts. With the cleaning procedure, the plants phenolic endogenous compounds were eliminated and the enzymes returned to their active form.

Table 3. Interference study for biosensors 1 (B1) and 2 (B2), using adrenaline solution 1.0×10^{-3} $mol \cdot L^{-1}$.

Interferents	Conc. 01(%)*		Conc. 02(%)**		Conc. 03(%)***	
	B1	B2	B1	B2	B1	B2
Ascorbic acid	15.15	–6.18	6.51	5.45	2.67	18.04
Noradrenaline	–11.85	–1.75	0.63	–2.45	6.63	11.16
Urea	2.99	–7.09	7.39	–2.19	–7.92	9.20
Uric acid	0.82	3.41	7.20	–1.51	0.46	–3.24
Dopamine	–5.49	6.38	–10.09	3.26	–0.57	5.89
Lidocaine	-	-	6.30****	5.40****	-	-

*Concentration below of physiological level in total blood; **Concentration normally equal to physiological levels in total blood; ***Concentration above of physyological levels in total blood; ****Concentration usually found in anesthetic medicines.

Table 4. Determination of adrenaline by UV-Spectrophotometry (Pharmacopoeia) and by proposed potentiometric biosensors.

Sample	Tagged value ($g \cdot L^{-1}$)	Pharmacopoeia ($g \cdot L^{-1}$)	Biosensor 1 ($g \cdot L^{-1}$)	Biosensor 2 ($g \cdot L^{-1}$)
1	0.010	0.010 ± 0.001	0.014 ± 0.009	0.011 ± 0.006
2	0.010	0.009 ± 0.003	0.013 ± 0.009	0.010 ± 0.007
3	1.000	1.082 ± 0.012	1.053 ± 0.063	0.953 ± 0.085

The main cleaning step for enzyme extraction advantage was the better biosensor selectivity. The biosensors with 125 $AU \cdot mL^{-1}$, containing cleaned enzymatic extracts, obtained using grinding, sonication for 30s, centrifugation, filtration and precipitation, had a good performance.

Potentiometric biosensor was applied in the epinephrine determination in local anesthetics for dental use and in adrenaline for clinical use. The method was efficient due to the analytical parameters obtained and the anesthetic non-interference, presenting as a viable alternative for adrenaline quality control in samples of pharmaceutical interest. Moreover, the tested substances interference, usually present in blood, was not as severe. Therefore, the method is feasible for in vivo analysis. The performance was satisfactory in precision and accuracy terms for adrenaline determination in drug samples.

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REFERENCES

[1] O. D. Leite, O. Fatibello-Filho and A. M. Barbosa, "Determination of Catecholamines in Pharmaceutical Formulations Using a Biosensor Modified with a Crude Extract of Fungi Laccase (*Pleurotus ostreatus*)," *Journal of the Brazilian Chemical Society*, Vol. 14, No. 2, 2003, pp. 297-303. doi:10.1590/S0103-50532003000200018.

[2] C. S. Magalhães, J. S. Garcia, A. S. Lopes, E. C. Figueiredo and M. A. Z. Arruda, "Strategies for Sample Preparation Focusing on Biomolecules Determination/Caracterization," In: M. A. Z. Arruda, Ed., *Trends in Sample Preparation*, Nova Science Publishers, New York, 2006, pp. 245-288.

[3] C. A. Signori, I. C. Vieira and O. Fatibello-Filho, "Quim. Nova," 1994, p. 38.

[4] A. W. Lima, V. B. Nascimento, J. J. Pedrotti and L. Angnes, "Coconut-Based Plant Tissue Reactor for Biosensing of Catechol in Flow Injection Analysis," *Analytica Chimica Acta*, Vol. 354, No. 1-3, 1997, pp. 325-331. doi:10.1016/S0003-2670(97)00428-5

[5] A. W. Lima, E. K. Vidsiunas, V. B. Nascimento and L. Angnes, "Vegetable Tissue from Latania sp.: An Extraordi-

nary Source of Naturally Immobilized Enzymes for the Detection of Phenolic Compounds," *Analyst*, Vol. 123, No. 11, 1998, pp. 2377-2382.

[6] O. Fatibello-Filho and I. C. Vieira, "Construction and Analytical Application of a Biosensor Based on Stearic Acid-Graphite Powder Modified with Sweet Potato Tissue in Organic Solvents," *Fresenius Journal of Analytical Chemistry*, Vol. 368, No. 4, 2000, pp. 338-343.

[7] O. Fatibello-Filho and I. C. Vieira, "Uso Analítico de Tecidos e de Extratos Brutos Vegetais Como Fonte Enzimática," *Química Nova*, Vol. 25, No. 3, 2002, pp. 455-464. doi:10.1590/S0100-40422002000300019

[8] P. R. B. O. Marques and H. Yamanaka, "Biosensors Based on the Enzymatic Inhibition Process," *Química Nova*, Vol. 31, No. 7, 2008, pp. 1791-1799. doi:10.1590/S0100-40422008000700034

[9] J. W. Anderson, "Extraction of Enzymes and Subcellular Organelles from Plant Tissues," *Phytochemistry*, Vol. 7, No. 11, 1968, pp. 1973-1988.

[10] A. M. Mayer and E. Harel, "Polyphenol Oxidases in Plants," *Phytochemistry*, Vol. 18, No. 2, 1979, pp. 193-215. doi:10.1016/0031-9422(79)80057-6

[11] A. M. Mayer, "Polyphenol Oxidases in Plants-Recent Progress," *Phytochemistry*, Vol. 26, No. 1, 1986, pp. 11-20. doi:10.1016/S0031-9422(00)81472-7

[12] L. R. V. Mataveli, N. J. Antunes, M. R. P. L. Brigagão, C. S. Magalhães, C. Wisniewski and P. O. Luccas, "Evaluation of a Simple and Low Cost Potentiometric Biosensor for Pharmaceutical and *in Vivo* Adrenaline Determination," *Biosensors and Bioelectronics*, Vol. 26, No. 2, 2010, pp. 798-802. doi:10.1016/j.bios.2010.06.021

[13] I. C. Vieira and O. Fatibello-Filho, "Biosensor Based on Paraffin/Graphite Modified with Sweet Potato Tissue for the Determination of Hydroquinone in Cosmetic Cream in Organic Phase," *Talanta*, Vol. 52, No. 4, 2000, pp. 681-689. doi:10.1016/S0039-9140(00)00420-3

[14] C. R. Dawson and W. B. Tarpley, "Copper Oxidases," In: J. B. Summer and K. Myrback, Eds., *The Enzymes—Chemistry and Mechanism of Action*, Academic Press, New York, 1951, pp. 455-491.

[15] L. N. Moreira, C. S. Magalhães and P. O. Luccas, "Emprego de Sistema e Análise em Fluxo Contínuo com Biossensor Potenciométrico para Determinação de Adrenalina em Medicamentos," *Infarma*, Vol. 16, No. 13-14, 2005, p. 59.

[16] L. S. Goodman and A. Gilman, "As Bases Farmacológicas da Terapêutica," 11th Edition, McGraw-Hill Interamericana do Brasil, Rio de Janeiro, 2006.

[17] S. S. Rosatto, R. S. Freire, N. Duran and L. T. Kubota, "Biossensores Amperométricos para Determinação de Compostos Fenólicos em Amostras de Interesse Ambiental," *Química Nova*, Vol. 24, No. 1, 2001, pp. 77-86. doi:10.1590/S0100-40422001000100014

[18] F. S. Felix, M. Yamashita and L. Angnes, "Epinephrine Quantification in Pharmaceutical Formulations Utilizing Plant Tissue Biosensors," *Biosensors and Bioelectronics*, Vol. 21, No. 12, 2006, pp. 2283-2289. doi:10.1016/j.bios.2005.10.025

[19] Q. L. Yang, P. Atasanov and E. Wilkins, "Needle-Type Lactate Biosensor," *Biosensors and Bioelectronics*, Vol. 14, No. 2, 1999, pp. 203-210. doi:10.1016/S0956-5663(98)00109-2

[20] I. Abdel-Hamid, P. Atanasov and E. Wilkins, "Development of a Needle-Type Biosensor for Intravascular Glucose Monitoring," *Analytica Chimica Acta*, Vol. 313, No. 1-2, 1995, pp. 45-54. doi:10.1016/0003-2670(95)00172-V

[21] E. C. Figueiredo, L. R. Souza, C. S. Magalhães, C. Wisniewski and P. O. Luccas, "A Homemade Autosampler/Injector Commutator for Flow Injection Analysis," *Journal of Automated Methods & Management in Chemistry*, Vol. 2006, 2006, Article ID: 42987.

[22] G. Li, B. J. Polk, L. A. Meazell and D. W. Hatchett, "ISE Analysis of Hydrogen Sulfide in Cigarette Smoke," *Journal of Chemical Education*, Vol. 77, No. 8, 2000, pp. 1049-1052.

[23] C. S. Caruzo, I. C. Vieira and O. Fatibello-Filho, "Determination of Epinephrine and Dopamine in Pharmaceutical Formulations Using a Biosensor Based on Carbon Paste Modified with Crude Extract of Cara Root (Dioscorea bulbifera)," *Analytical Letters*, Vol. 32, No. 1, 1999, pp. 39-50. doi:10.1080/00032719908542597

[24] C. S. Magalhães and M. A. Z. Arruda, "Sample Preparation for Metalloproteins Analysis: A Case Study Using Horse Chestnuts," *Talanta*, Vol. 71, No. 5, 2007, pp. 1958-1963. doi:10.1016/j.talanta.2006.08.039

[25] M. Becerra, E. Rodríguez-Belmonte, M. A. Cerdán and M. I. G. Siso, "Extraction of Intracellular Proteins from Kluyveromyces Lactis," *Food Technology and Biotechnology*, Vol. 39, No. 2, 2001, pp. 135-139.

[26] C. C. Nascentes, M. Korn, C. S. Sousa and M. A. Z. Arruda, "Use of Ultrasonic Baths for Analytical Applications: A New Approach for Optimisation Conditions," *Journal of the Brazilian Chemical Society*, Vol. 12, No. 2, 200, pp. 157-163.

[27] K. Kabaha, A. Taralp, I. Cakmak and L. Ozturk, "Accelerated Hydrolysis Method to Estimate the Amino Acid Content of Wheat (Triticum Durum Desf.) Flour Using Microwave Irradiation," *Journal of Agricultural and Food Chemistry*, Vol. 59, No. 7, 2011, pp. 2958-2965.

[28] L. Long, P. Wu, X. Guan, J. Zhang, Y. Jin, Z. Zhang, Y. Wang, Y. Li, J. Chen and W. Wang, "Determination of Protein-Bound Methionine Oxidation in the Hippocampus of Adult and Old Rats by LC-ESI-ITMS Method after Microwave-Assisted Proteolysis," *Applied Biochemistry and Biotechnology*, Vol. 399, No. 6, 2011, pp. 2267-2274.

[29] S. Shin, A. Lee, S. Lee, K. Lee, J. Kwon, M. Y. Yoon, J. Hong, D. Lee, G. Lee and J. Kim, "A Label-Free Biosensor Assay for Botulinum Neurotoxin B in Food and Human Serum," *Analytical Biochemistry*, Vol. 407, No. 2, 2010, pp. 281-288. doi:10.1016/j.ab.2010.08.021

[30] H. Zheng, J. Yin, Z. Gao, H. Huang, X. Ji and C. Dou, "Disruption of Chlorella Vulgaris Cells for the Release of Biodiesel-Producing Lipids: A Comparison of Grinding, Ultrasonication, Bead Milling, Enzymatic Lysis, and Microwaves," *Applied Biochemistry and Biotechnology*, Vol. 164, No. 7, 2011, pp. 1215-1224.

doi:10.1007/s12010-011-9207-1

[31] H. P. Harper, "Manual de Química Fisiológica," 3rd. Edition, Atheneu, São Paulo, 1973.

[32] M. M. Bradford, "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding," *Analytical Biochemistry*, Vol. 72, No. 1-2, 1976, pp. 248-254. doi:10.1016/0003-2697(76)90527-3

[33] D. A. M. Zaia, C. T. B. V. Zaia and J. Lichtig, "Determinação de Proteínas Totais via Espectrofometria: Vantagens e Desvantagens dos Métodos Existentes," *Química Nova*, Vol. 21, No. 6, 1998, pp. 787-793. doi:10.1590/S0100-40421998000600020

[34] S. J. Compton and C. G. Jones, "Mechanism of Dye Response and Interference in the Bradford Protein Assay," *Analytical Biochemistry*, Vol. 151, No. 2, 1985, pp. 369-374. doi:10.1016/0003-2697(85)90190-3

[35] L. R. V. Mataveli, "Medida de Atividade Enzimática de Extrato Bruto de Banana (Musa sp.): Comparação Entre Diferentes Metodologias," *Infarma* (*Brasília*), Vol. 19, No. 9-10, 2007, pp. 33-35.

[36] J. C. S. Barboza and A. A. Serra, "Ultra-Som I: Influencia do Ultra-Som Na Quimica," *Química Nova*, Vol. 15, No. 4, 1992, pp. 302-316.

[37] R. P. Buck and E. Lindner, "Recommendations for Nomenclature of Ionselective Electrodes (IUPAC Recommendations 1994)," *Pure and Applied Chemistry*, Vol. 66, No. 12, 1994, pp. 2527-2536. doi:10.1351/pac199466122527

[38] E. S. Louzada, P. O. Luccas and C. S. Magalhães, "Construção e Caracterização de um Biossensor Potenciométrico Para Determinação de Pirogalol," *Revista Analytica*, Vol. 11, 2004, pp. 52-57.

[39] C. A. S. Perone, M. P. Capobianco and S. P. Junior, "Kinetic Characterization of Enzyme Polyphenol Oxidase, Utilizing Crude Extract of Pell of Stunded Banana (Musa Acuminata)," *Revista do Instituto de Ciências da Saúde*, Vol. 26, No. 2, 2008, pp. 201-206.

[40] A. A. M. Chasin, M. Chasin and M. C. Salvadori, "Validação de Métodos Cromatográficos em Análises Toxicológicas,"*Revista Brasileira de Toxicologia*, Vol. 11, 1998, pp. 1-6.

[41] "Farmacopéia Brasileira," 3rd Edition, São Paulo, 1977.

[42] D. A. Skoog, F. J. Holler and T. A. Nieman, "Principles of Instrumental Analysis," 5th Edition, Bookman Ed., São Paulo, No. 1, 2006.

