Brazilian Journal of Analytical Chemistry
2024, Volume 11, Issue 43, pp 64-77
doi: 10.30744/brjac.2179-3425.AR-105-2023

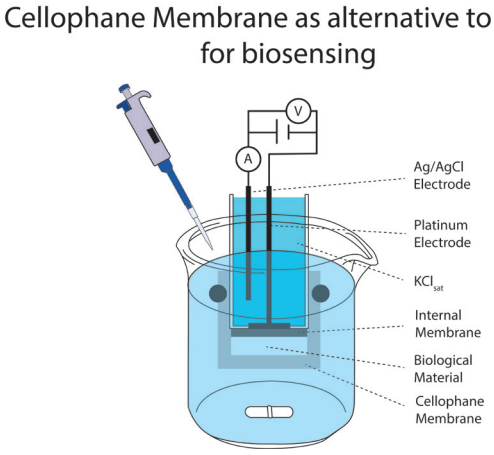
BrJAC

ARTICLE

Development and Performance Evaluation of Low-Cost Cellophane Paper-Based Biosensors for Polyphenol Detection in Teas: A Cost-Effective Alternative to Teflon® Membranes Biosensors

Cássia Aparecida Signori Perone, Thiago Regis Longo Cesar Paixão*

Departamento de Química Fundamental do Instituto de Química da Universidade de São Paulo
Avenida Professor Lineu Prestes, 748, Butantã, 05508-900, São Paulo, SP, Brazil



This study introduces biosensors developed using cellophane paper with immobilized polyphenol oxidase (PPO) from dwarf banana and eggplant peels, integrated with glutaraldehyde and a Clark electrode. These biosensors, optimized for enzyme concentration at 75 units for dwarf banana peel and 100 units for eggplant peel, demonstrated high sensitivity to catechol, paracetamol, pyrogallol, and hydroquinone, similar to traditional Teflon® Clark electrodes. Despite the lower measurement capacity of cellophane membranes (20-30) compared to Teflon's (300-400), the cost-effectiveness and accessibility of cellophane offer a strategic advantage. These biosensors effectively quantified polyphenols in teas, with results closely matching those from the standard Folin-Dennis method and Teflon® electrodes, maintaining a 95% confidence level and an error margin of less than 1%. Hence, the cellophane paper-based biosensors present a cost-effective, efficient alternative for polyphenol detection in teas, promising broader application due to their affordability and performance.

Keywords: Polyphenol oxidase, cathecol, eggplant peels, dwarf banana peels, Clark electrode, low-cost phenol sensing

INTRODUCTION

Selectivity is one of the biggest problems in analytical chemistry, and it is usually obtained only through intense control of the experimental conditions. However, in nature, highly selective structures are found in enzymes, antibodies, and others. Enzymes, in particular, have several advantages, such as a joyful combination of selectivity with sensitivity, allowing the use of various transduction technologies.[1] Current scientific publications describe various devices that exploit enzymes (biological material) in combination with electrochemical transducers.[2-6]

Cite: Perone, C. A. S.; Paixão, T. R. L. C. Development and Performance Evaluation of Low-Cost Cellophane Paper-Based Biosensors for Polyphenol Detection in Teas: A Cost-Effective Alternative to Teflon® Membranes Biosensors. *Braz. J. Anal. Chem.* 2024, *11* (43), pp 64-77. http://dx.doi.org/10.30744/brjac.2179-3425.AR-105-2023

Submitted 24 November 2023, Resubmitted 23 January 2024, Accepted 24 January 2024, Available online 1th February 2024.

In Brazil, federal legislation establishes a differentiated maximum limit of total phenols concerning the disposal of effluents in bodies of water. This resolution imposes a maximum concentration of 0.5 mg L^{-1} of phenols.[7] Phenolic compounds, abundant secondary metabolites in the plant kingdom, are extensively studied for their diverse roles. These compounds primarily serve as defense mechanisms in fruits and vegetables, imparting astringency, color, flavor, and aroma. In humans, consuming these compound-rich fruits and vegetables is beneficial mainly due to their potent antioxidant properties, which safeguard against oxidative stress and aid in reducing inflammation and preventing cardiovascular diseases, certain cancers, and premature aging. Interestingly, many medicinal drugs are derived from plants rich in phenolic compounds. For instance, *Cannabis sativa* is notable for its high phenolic content, utilized in controlled doses for the treatment of behavioral disorders under professional guidance. However, it is crucial to acknowledge the potential toxicity of these compounds; unregulated consumption can lead to adverse effects such as liver damage. In the pharmaceutical industry, even synthetic drugs like paracetamol, containing a phenolic group, demonstrate the dual nature of these compounds – acting as effective analgesics at prescribed doses while posing risks of severe liver damage when consumed excessively. Similarly, hydroquinone, used in chemical peels, exemplifies the potential for skin damage and high toxicity.

This underscores the critical need for efficient, rapid, and sensitive methods to quantify phenolic compounds, balancing their beneficial uses against potential risks. Thus, growing investment in developing technologies that carry out this monitoring, such as biosensors, is promissory. Biosensors reveal the great potential for monitoring phenol compounds, enabling speed and efficiency.[8] They could be advantageous to be low-cost depending on the enzyme source and immobilization process. In addition, they have unique characteristics, such as selectivity, low cost of construction, storage, and detection limit; ease of automation; and construction of simple and portable equipment for on-site monitoring.[9]

The incorporation of molecules with biological activity has significantly increased the development of these biosensors using the enzyme polyphenol oxidase (PPO) extracted from crude extracts of vegetables and fruits,[2,10-14] for quantification of these analyses in different applications for environmental, pharmaceutical, and food areas and using expensive substrates to immobilize the biologically active molecule. Related to PPO enzyme sources, we can cite works in 2015 by Ribeiro et al.[15] developed a yam biosensor for detecting phenols in wastewater. Vega and collaborators[16] built a nanosensor for detecting phenols, analyzing wastewater in agriculture, and containing polyphenols from fertilizers and pesticides. Sousa and collaborators[17] built a portable voltammetric biosensor using Wi-Fi technology to analyze phenolic compounds in an urban environment using a biosensor composed of three microelectrodes, with the PPO pure enzyme placed on the working microelectrode, and the sample to be analyzed needs to come into contact with the three microelectrodes and the enzyme.

In summary, the literature has shown the development of biosensors using PPO enzyme and Teflon® membrane to immobilize the enzyme with glutaraldehyde (a polymerizing agent)[18-21] is an interesting combination. However, there are still challenges to effectively commercializing biosensors, and sometimes, it is related to the substrate for the immobilization of the enzyme. Hence in the present work, the enzyme PPO from crude extracts was immobilized on a cellophane paper, a low-cost material compared to Teflon®, with glutaraldehyde. The advantage of the proposed developed method is based on reducing the cost and complexity in the construction of the biosensor without loss of stability, response time, selectivity, and detection limit. The method for quantifying and immobilizing is straightforward, involving a single-step process where polymerization and enzymatic immobilization happen concurrently. PPO facilitates the conversion of monophenols and diphenols into quinones, utilizing molecular oxygen in the oxidation process.[22] The quinones formed in this reaction polymerize to form melanins.[22] Thus, the concentration of phenolic compounds will decrease the oxygen concentration in the samples measured with a Clark-type oxygen electrode.

MATERIALS AND METHODS

Equipment

To obtain the raw extracts of eggplant and dwarf banana peels in 0.1 mol L^{-1} phosphate buffer solutions of pH 6.5,[23] a crusher (blender) and an Analyzer model 300 pH meter in addition to a centrifuge from Du Pont Instruments Sorvael, model RC-5B Plus. An Analyzer Orion model VIS 7220 spectrophotometer was used to determine the extracts' enzyme activity and total protein. The Clark-type electrode and the oxygen analyzer used to study biosensors are from DIGIMED, model DM-CO1 (electrode), and model DM-4 V5 (analyzer).

Reagents

Polyclar SB-100 (Indústria ISP do Brasil Ltda), Polyvinylpyrrolidone (PVP), was used to remove excess natural polyphenols in the crude extracts studied through hydrogen bonding. It stabilizes juices, wines, and beer, insoluble in water, presenting easy removal. Analytical grade reagents were used to prepare 0.1 mol L^{-1} phosphate buffer solutions (pH 6.0-7.5), 0.1 mol L^{-1} acetate buffer solutions (pH 3.0-5.5), and catechol and pyrogallol standard solutions (Sigma Chemical Co-St. Louis, Mo, USA), paracetamol, and hydroquinone (Reagen, Laboratory Products, Paraná, Brazil), which was prepared daily. Bovine serum albumin solution (1%) m/V (Sigma Chemical Co) was used as a standard in protein analysis. The glutaraldehyde solution (2.5%) m/V (Reagen, Laboratory Products, Paraná, Brazil) will be used together with a cellophane paper membrane (acquired in the packaging market) as a support for the enzyme immobilization. Samples of some types of filter bag teas, such as mate, black, chamomile, mint, fennel, and green from the Oetker brand, were used to quantify polyphenols by the amperometric biosensors produced by dwarf banana and eggplant peels. All tea samples were obtained from the local market. Some additional information about the composition of the teas used:

- Black tea: high content of flavonoids, polyphenols, tannins, caffeine, theophylline, and B complex vitamins.
- Mate tea: caffeine, alkaloids, proteins, carbohydrates and lipids.
- Chamomile tea: Chamazulene, α-bisabolol and α-bisaboloxides.
- Mint tea: menthol, ascorbic acid, flavonoids and terpenes.
- Green tea: pigments and carbohydrates.
- Fennel tea: coumarin and malic acid.

The tannic acid solution (0.1 mg L^{-1}) and saturated sodium bicarbonate (Reagen) were prepared for spectrophotometric analysis of total polyphenols and the Folin-Denis reagent for the standard curve of the tea samples.

Analytical procedure

Biological Material

Eggplants and dwarf bananas were purchased in the local market (250 g each), approximately two eggplants and 2 dwarf bananas.

Extraction of the enzyme Polyphenol oxidase from eggplant and banana peels

Twenty-five grams of each biological material were peeled into small pieces and homogenized in a blender with 50 mL of 0.1 mol L^{-1} phosphate buffer solution, pH 6.5, containing 2.5 g of Polyclar for the eggplant pee[24] and 7.5 g of Polyclar for dwarf banana peel.[23] Then, they were centrifuged at 14000 rpm for 20 minutes. The supernatant solutions were stored in a freezer as a source of PPO enzyme for activity measurements, total protein, and biosensor construction.[13]

Determination of polyphenol oxidase activity in crude extracts

The activity of soluble PPO, found in biological substances, was determined by measuring the absorbance at a wavelength of 410 nm. This measurement corresponds to the melanin produced from

the polymerization of quinine, which occurs after a reaction. This reaction involves mixing 0.2 mL of the supernatant solution, derived from each extract mentioned previously, with 2.8 mL of catechol in a 0.1 mol L^{-1} phosphate buffer solution at a pH of 6.5 and a temperature of 25 °C.[14] The reaction was monitored for 2 minutes,[25] the time necessary to reach V_{max}. The activity unit is described as the quantity of enzyme necessary to produce a 0.001 increase in absorbance units per minute, following the conditions outlined in Equation 1.

$$a = \frac{\Delta A.\,60.1000}{\Delta t.\,d.\,V_{sample}} \qquad \text{Equation 1}$$

where: a = activity / U mL^{-1}; ΔA = change in absorbance; Δt = time variation / min; d = diameter of the cuvette; V_{sample} = sample volume / mL.

The total supernatant protein solutions of the crude extracts were quantified by the biuret method[25] using bovine serum albumin as a standard.

Biuret solution for dosage of total protein in the studied crude extracts

The solutions were prepared by adding 1.50 g of $CuSO_4.5H_2O$ and 6.0 g of sodium potassium tartrate separately dissolved in 500 mL of distilled water. With constant stirring, 300 mL of 10% NaOH was added. It was diluted to 1 L with distilled water and stored in a refrigerator. This solution is generally stable for over 1 year, but if a red precipitate of CuO appears, it is discarded and prepared again.

Standard solution of bovine serum albumin protein (1%) m/V

A 1% w/v bovine serum albumin solution (Sigma Chemical Co) was used, and it was prepared in 0.1 mol L^{-1} phosphate buffer solution, pH 6.5.

Method of immobilizing polyphenol oxidase (PPO) enzyme with glutaraldehyde bifunctional reagent using cellophane paper and biosensor preparation

A cellophane paper was used to support the immobilization of PPO present in crude extracts of dwarf banana and eggplant peels. The circular areas of the membranes were covered, with different amounts of units of PPO solutions, from the crude extract of dwarf banana peel (25 U; 50 U; 75 U, and 100 U) and the crude extract of eggplant peel (50 U; 75 U; 100 U and 150 U) with glutaraldehyde 2.5% w/v, in the same proportion.[2] The membranes were dried in a desiccator at 25 ºC for 10 hours.

These polymerization step, particularly using glutaraldehyde, is crucial in our biosensor design for several reasons. Primarily, it promotes the covalent bonding of the enzyme protein to the membrane surface, leading to the enzyme's insolubilization. This immobilization is essential as it retains the enzyme's catalytic activity while preventing its dissolution into the solution during the biosensor's operation. Furthermore, the use of glutaraldehyde does not only immobilize the enzyme but also forms a gel-like layer on the membrane's surface. This gel layer is significant for creating a stable microenvironment around the enzyme, which can enhance the overall stability and activity of the biosensor. The gel matrix allows for a controlled diffusion of analytes to the enzyme's active sites, which is crucial for consistent and reproducible sensor responses.

Two minutes before the determinations, they were introduced into a 0.1 mol L^{-1} phosphate buffer solution, pH 6.5, at a temperature of 4 ºC to carry out the amperometric measurements. To perform the amperometric measurements, the membranes were placed at the end of the oxygen electrode and fixed with a rubber ring (Figure 1).

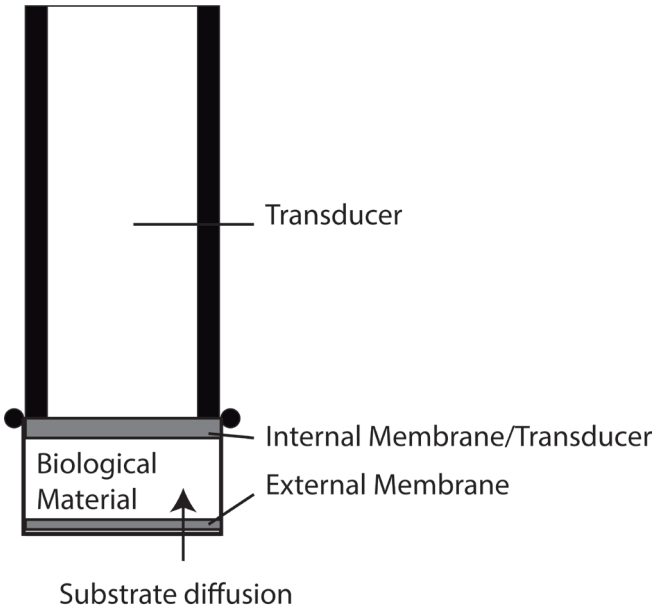


Figure 1. Schematic representation of the experimental set-up.

The biosensor, which consists of an oxygen electrode equipped with each created enzymatic membrane, was positioned in a glass cell. This cell contained 50 mL of phosphate buffer solution with a concentration of 0.1 mol L^{-1} and a pH of 6.5, maintained at a temperature of 25 ºC. After stabilization, 1.0 µL of catechol solution 5.0×10^{-5} mol L^{-1} is added with constant stirring.

The biosensors were used for amperometric measurements to quantify polyphenols in some tea samples. For comparison, Teflon® membranes (Celgard 2400) were fabricated using the same procedure, changing the cellophane paper for Teflon® membranes.

Amperometric quantification of phenolic substrates concentration

The biosensor, which consists of an oxygen electrode equipped with each created enzymatic membrane, was positioned in a glass cell. This cell contained 50 mL of phosphate buffer solution with a concentration of 0.1 mol L^{-1} and a pH of 6.5, maintained at a temperature of 25 ºC. After stabilization, increasing volumes of 10 mmol L^{-1} catechol substrate (10-200 µL) were added at 1-minute intervals between each addition, with constant agitation. The addition of the substrate causes a decrease in the O_2 concentration with the consequent decrease in the reduction current of this species.[1] The sample concentration containing polyphenols was determined by the same procedure, using the analytical curve thus obtained (a curve was plotted for each sample studied).

Effect of immobilized polyphenol oxidase concentration on biosensors

The crude extracts of eggplant and banana peels studied showed the best source of the enzyme PPO; thus, they were selected for this project.[23,24] The effect of the concentration of PPO (units) immobilized on the cellophane paper matrix was studied, as indicated in “Method of immobilizing polyphenol oxidase (PPO) enzyme with glutaraldehyde bifunctional reagent using cellophane paper and biosensor preparation”, concerning the response of these biosensors and/or stability.

Effect of pH on the response of amperometric biosensors

The effect of pH on the biosensor response of eggplant and dwarf banana peels was studied in the pH range ranging from 3.0-7.5; using the catechol substrate at a concentration of 5.0×10^{-5} mol L^{-1}.[1]

Determination of response time for the studied biosensors

The response time for the biosensors of eggplant and dwarf banana skins was determined using catechol 5.0 × 10^{-5} mol L^{-1} as substrate, and the number of determinations performed for each biosensor was also studied.

Relative response of biosensors to different phenolic substrates

The relative response to the biosensors of eggplant and dwarf banana skins was determined using phenolic substrates such as catechol, pyrogallol, paracetamol, and hydroquinone (2.5 ×10^{-3} mol L^{-1}) in 0.1 mol L^{-1} phosphate buffer pH 6.5.[23,26]

Preparation of tea samples

The samples were prepared by infusing 10 g of tea in 15 mL of double-distilled water (previously heated, 80 ºC). The infusion time was 10 minutes, after this infusion period, the temperature of the solution was approximately 40 ºC. Then, this solution containing the tea was placed in a volumetric flask, and the volume was made up to 25 mL with 0.1 mol L^{-1} phosphate buffer solution, pH 6.5.

Determination of total Folin-Denis phenols (Standard Spectrophotometric Method) for polyphenols in tea samples

The Folin-Denis reagent was prepared as described in the literature.[27] The standard curve is prepared using aliquots of 0-10 mL of tannic acid (0.1 mg L^{-1}), 5 mL of Folin-Denis reagent, and 10 mL of sodium bicarbonate (saturated solution) in a 100 mL flask. After 30 minutes, the absorbance at 760 nm is determined.

The blue-green color produced in this method results from a colorimetric reaction involving the reduction of phosphomolybdic-phosphotungstic acid by phenolic compounds. The intensity of the color produced depends on the phenolic compounds amount present in the sample.

RESULTS AND DISCUSSION

Determination of polyphenol oxidase activity and total protein in crude extracts of dwarf banana and eggplant peels

Crude extracts from banana and eggplant peels were studied as biocatalytic materials for the oxidation of the catechol phenolic substrate. Table I shows the activities (U mL^{-1}) found in these extracts, total protein (mg mL^{-1}), and specific activity (U mg^{-1} of total protein). As we can see in this table, the specific activity of PPO in the crude extract of dwarf banana peel was better than in eggplant peel; however, compared to the purified enzyme, both showed higher activity and could be used for biosensor fabrication. The stability times of crude extracts of dwarf banana and eggplant peels about PPO enzyme were studied being stored in a freezer, it was 40 days for the banana peel and 34 days for the eggplant peel, being able to work at temperatures close to 35 ºC for the two extracts, without significant loss of enzyme activity.

Table I. Activity, total protein and specific activity of polyphenol oxidase in crude extracts of dwarf banana and eggplant peels

Material	Activity (U mL^{-1})	Total Protein (mg mL^{-1})	Specific Activity (U mg^{-1} of protein)
Crude extract of dwarf banana peel	30900	24.0	1287.5
Crude extract of eggplant peel	22350	27.5	812.8
Pure Enzyme*	-	-	2400
Purified** Enzyme mushroom	1075	23.0	47

* Sigma The linear regression for the standard curve of bovine serum albumin for the dosage of total protein in the two extracts studied was Absorbance = 0.00548 + 0.03507 (Protein mass/mg); **Ref. No. 28.

Effect of polyphenol oxidase concentration of crude extracts studied on cellophane paper biosensor and comparison with Teflon® membrane

Crude extract of dwarf banana peel

The study of the effect of PPO concentration of crude extracts on cellophane paper biosensors and comparison with Teflon membrane using Clark´s electrode showed that the biosensor of crude extract of dwarf banana peel in cellophane paper behaves similarly to the biosensor built-in Teflon (Figure 1), with respect to the concentration of immobilized enzyme (75 U).

Hence, there is an advantage in using cellophane paper because the cost-benefit in relation to Teflon is much higher (cellophane paper 1 dollar per meter while a 0.5 cm diameter Teflon membrane costs 88 dollars). The biosensor response increases with increasing concentration of the enzyme immobilized on the outer membrane of the oxygen electrode up to 75 U for both cases, decreasing next (Figure 2). This could be attributed to the thickening of the enzymatic layer, which hampers the transport of both the substrate and molecular oxygen, consequently leading to a reduction in oxygen consumption.

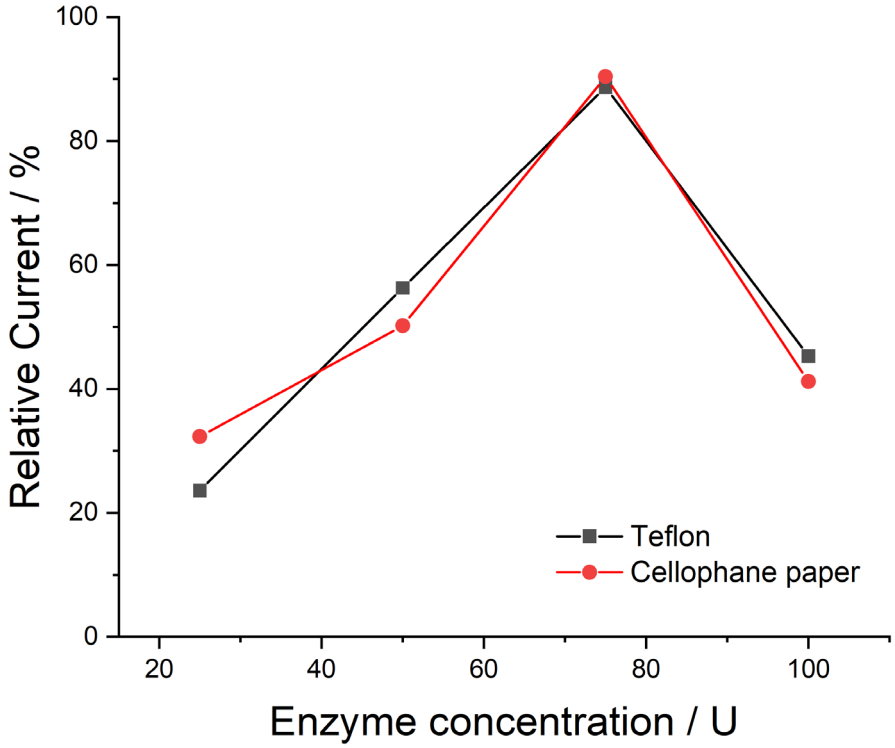


Figure 2. Effect of polyphenol oxidase enzyme concentration (U) on the relative current response [If/Ii × 100] of the biosensor obtained from crude dwarf banana peel extract in phosphate buffer solution, pH 6.5, at 25 ºC. Where If = % final current and Ii = % initial current. Conditions: substrate catechol 5×10^{-5} mol L^{-1}, in Teflon membrane and cellophane paper.

Crude eggplant peel extract

The biosensor built from eggplant skin on cellophane paper behaves better than the biosensor built on Teflon, as the amount of immobilized enzyme, that is, in cellophane paper (100 U) was lower than in Teflon (150 U). The greater permeability of cellophane can explain this compared to Teflon, which may also include the material´s thickness. With this, there is an advantage in using cellophane paper because the cost-benefit of Teflon is much higher. The biosensor response increases with increasing concentration of the enzyme immobilized on the outer membrane of the oxygen electrode up to 100 U for cellophane,

decreasing next (Figure 3). This phenomenon might be linked to the enhanced thickness of the enzymatic layer, which impedes the movement of the substrate and molecular oxygen, resulting in a decreased consumption of oxygen.

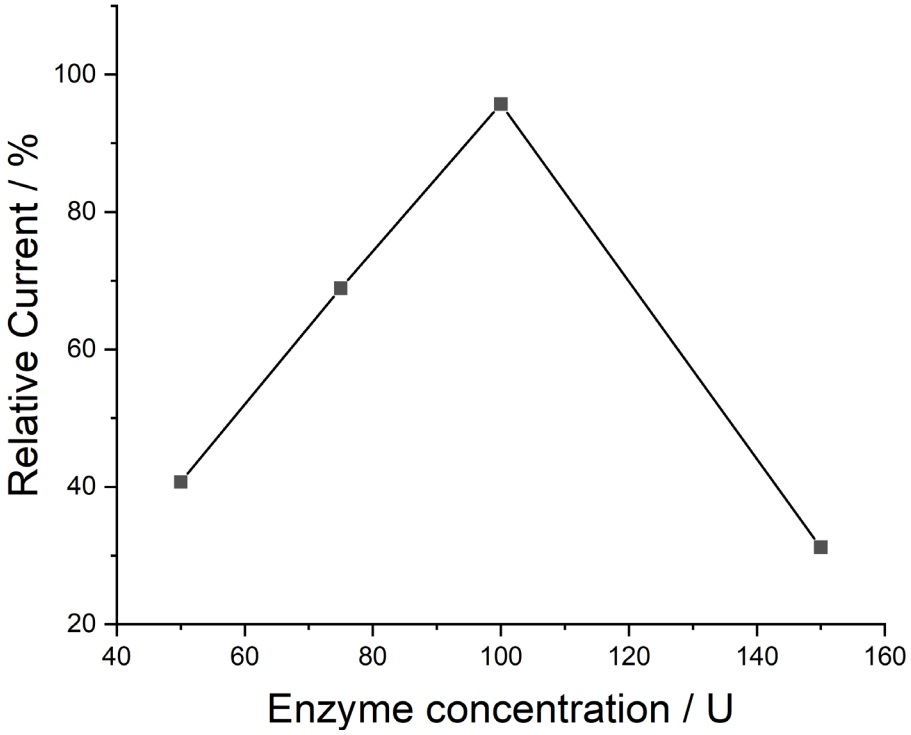


Figure 3. Effect of polyphenol oxidase enzyme concentration (U) on the response [$I_f/I_i \times 100$] of the crude eggplant peel extract biosensor in phosphate buffer solution, pH 6.5, at 25 ºC, for the substrate 5×10^{-5} mol L^{-1} catechol, in cellophane membrane. Where I_f = % final current and I_i = % initial current.

Effect of pH on the response of amperometric biosensors of crude extracts of dwarf banana and eggplant peels in cellophane paper

The study of the effect of pH (3.0-7.5) on the response of biosensors from crude extracts of dwarf banana peel and eggplant peel to the catechol substrate 5.0×10^{-5} mol L^{-1} was determined using a cellophane membrane with 75 Units of Polyphenol oxidase (PPO) for dwarf banana peel and 100 U for eggplant peel, at 25 ºC. It used 0.1 mol L^{-1} acetate buffer solution (pH 3.0-5.5) and 0.1 mol L^{-1} phosphate buffer solution (pH 6.0-7.5).

The best immobilization pH on the responses of biosensors of crude extract of dwarf banana peel and eggplant peel was 6.5 (Figures 4 and 5). It used 0.1 mol L^{-1} phosphate buffer solution (same values found with the enzyme immobilized on the Teflon membrane as in Signori and Fatibello-Filho,[14] evidencing that cellophane works as well as Teflon immobilization support for PPO in the two extracts studied.

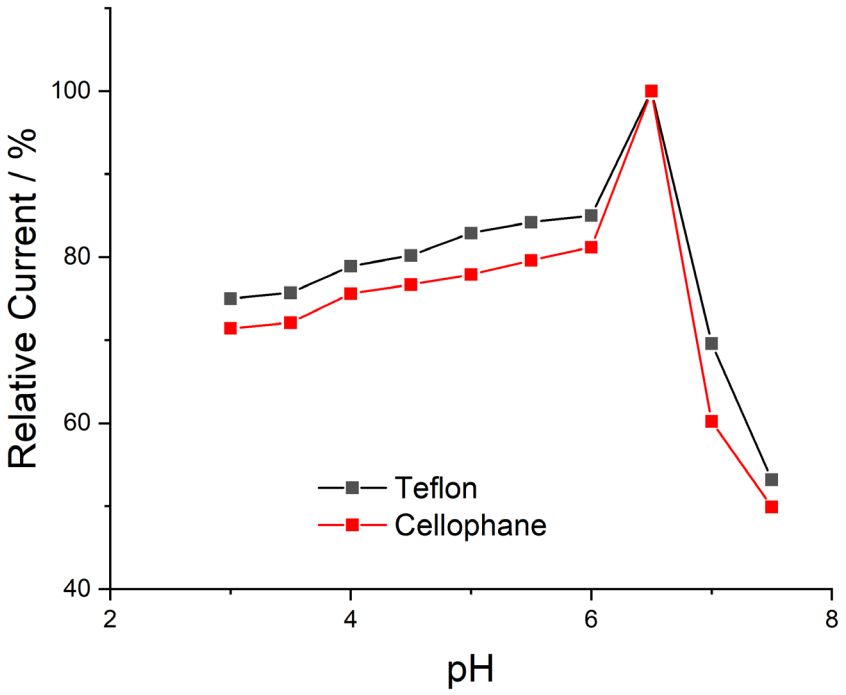


Figure 4. Effect of pH on the response [I_f/I_i x100] of the dwarf banana peel biosensor, at 25 ºC, for the catechol substrate 5×10^{-5} mol L^{-1}, using 0.1 mol L^{-1} acetate buffer solution (pH 3.0-5.5) and 0.1 mol L^{-1} phosphate buffer solution (pH 6.0-7.5). Where I_f = % final current and I_i = % initial current.

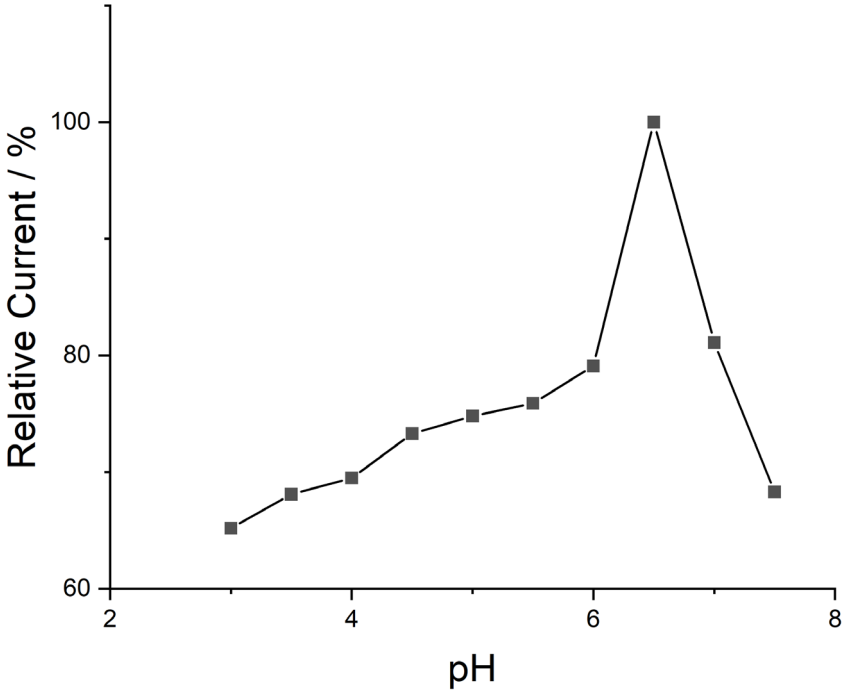


Figure 5. Effect of pH on the response [$I_f/I_i \times 100$] of the eggplant skin biosensor, at 25 ºC, for the catechol substrate 5×10^{-5} mol L^{-1}, using 0.1 mol L^{-1} acetate buffer solution (pH 3,0-5.5) and 0.1 mol L^{-1} phosphate buffer solution (pH 6.0-7.5) on cellophane paper. Where I_f =% final current and I_i =% initial current.

Response time study for the biosensors of crude extracts of dwarf banana and eggplant peels

The response time for the banana peel biosensor was shorter using cellophane paper (40 seconds) as support for immobilization than for Teflon 58 seconds.[14,23] The same can be observed for the eggplant skin biosensor in cellophane paper 45 seconds and in Teflon 64 seconds. This can be explained because cellophane has a thinner membrane thickness than Teflon, facilitating the diffusion of the substrate to the biosensor and consequently decreasing the response time. This study was also performed using two membranes with immobilized enzymes coupled to the amperometric detector instead of just one; the response time was approximately 5 minutes, showing the impossibility of using more than one membrane. Therefore, it can be concluded that with two membranes, the phenolic substrate cannot diffuse to the detector, making the quantification impossible.

Interfering species test: Relative response of biosensors of crude extracts of dwarf banana and eggplant peels in cellophane membrane for different phenolic substrates

Tables II and III show the response of cellophane membrane biosensors to these phenolic substrates: catechol, pyrogallol, paracetamol, and hydroquinone. Biosensors were more sensitive to catechol, followed by paracetamol, pyrogallol, and hydroquinone; evidencing catechol as the main substrate for determining the activity of PPO and the calibration curves of biosensors for determinations of polyphenols. The biosensors studied with cellophane agreed regarding the relative response for different phenolic substrates with the biosensors studied by Signori and Fatibello-Filho,[14] using The Teflon membrane as an immobilization matrix. Thus, it can be used to determine polyphenols instead of the Teflon biosensor; because it has low cost and easy access (national product).

Table II. Relative response of the biosensor of crude extract of dwarf banana peel in cellophane paper, using phenolic substrates (5×10^{-3} mol L^{-1}) in phosphate buffer solution 0.1 mol L^{-1} pH 6.5 at 25 ºC

Phenolic Substrates (5×10^{-3} mol L^{-1})	Relative current (%)
Catechol	*100*
Paracetamol	99.8
Pyrogallol	98.7
Hydroquinone	98.3

Where: [I_f/I_i x 100] = % relative current

Table III. Relative biosensor response of crude extract of eggplant peel in cellophane paper, using phenolic substrates (5×10^{-3} mol L^{-1}) in phosphate buffer solution 0.1 mol L^{-1} pH 6.5 at 25 ºC

Phenolic Substrates (5×10^{-3} molxL^{-1})	Relative current (%)
Catechol	*100*
Paracetamol	99.5
Pyrogallol	97.2
Hydroquinone	97.1

Where: [I_f/I_i x 100] = % relative current

Study of the number of determinations for the biosensors of crude extracts of dwarf banana and eggplant peels in cellophane membrane as immobilization matrix

With each enzyme membrane of the banana peel electrode with polyphenol oxidase immobilized on cellophane paper, it was possible to make an average of approximately 30 determinations, and with the eggplant peel electrode, around 20 determinations, resulting in two days of use for each studied electrode. The membranes with the immobilized enzyme must not be removed from the surface of the oxygen electrode during this determination. They must be kept in 0.1 mol L^{-1} phosphate buffer solution, pH 6.5 at 25 ºC during and after its use. This can be explained due to the low resistance of this membrane in its handling; that is, if it is removed from the surface of the oxygen electrode, it tends to be damaged, making further determinations impossible. Comparing the Teflon membranes (300-400 determinations and approximately 30 to 40 days of use for the electrodes of dwarf banana and eggplant peels, with cellophane membranes, we can verify that the latter is less resistant, having to be replaced more quickly than the Teflon membrane. We can then conclude that we have practically disposable enzymatic electrodes if the matrix is made of cellophane paper. Due to the ease of obtaining this polymer (national product) and its low cost compared to Teflon (imported product), we can build several membranes with cellophane paper and obtain advantages in the determinations before Teflon.

Quantifying polyphenols in tea samples, with biosensors from crude extracts of dwarf banana and eggplant peels using cellophane paper as a support, for the immobilization of Polyphenol oxidase present in these extracts

To evaluate the performance of these biosensors, the content of polyphenols (mg g^{-1} of dry tea) was determined in samples of teas (mate, black, chamomile, mint, fennel, and green). Tables IV and V below compare the polyphenol contents obtained by the proposed amperometric method and the spectrophotometric (standard).[29]

The levels of polyphenols were found to agree with those obtained by the standard method at a confidence level of 95% and within an acceptable error range (< 1%), evidencing the efficiency of the developed method. The limit of quantification (LOQ) for our biosensor method has been determined to be 10^{-5} mol L^{-1}. This was established in a 0.1 mol L^{-1} phosphate buffer solution at pH 6.5 and a temperature of 25 ºC. These details are in Tables II and III, where we present the data supporting our LOQ determination.

The LOD has been calculated to be 10-6 mol L^{-1}, representing the lowest analyte concentration in a sample that can be reliably detected, not merely distinguished from zero. This has been determined through triplicate measurements, with the standard deviation and relative error presented in Tables IV and V.

The biosensors studied presented a good analytical performance with smaller relative errors and more significant results than the biosensors of banana peel and eggplant built with Teflon.[23-25] After 2 days of use in both biosensors, it was observed that there was a significant loss of immobilized material (lyophilization).

Table IV. Determination of polyphenols in tea samples using the amperometric method proposed and the standard spectrophotometric method,[29] with a biosensor of crude extract of polyphenol oxidase from the dwarf banana peel (*Musa acuminata*) on cellophane paper

Samples	Phenol concentration (mg g^{-1} dry tea) Spectrophotometric	Phenol concentration (mg g^{-1} dry tea) Amperometric	Relative Error (%)
Yerba mate tea	2.80 ± 0.02*	2.78 ± 0.01	- 0.71
Black tea	2.91 ± 0.01	2.89 ± 0.03	- 0.69
Chamomile tea	1.95 ± 0.03	1.94 ± 0.04	- 0.51
Mint tea	0.17 ± 0.02	0.17 ± 0.01	0.00
Fennel tea	< LOQ	< LOQ	-
Green tea	< LOQ	< LOQ	-

* standard deviation of the mean with a confidence level of 95%, for n=3.

Table V. Determination of polyphenols in tea samples using the amperometric method proposed and the standard spectrophotometric method,[29] with a biosensor of crude extract of polyphenol oxidase from eggplant peel (*Solanum melongena L.*) on cellophane paper

Samples	Phenol concentration (mg g^{-1} dry tea) Spectrophotometric	Phenol concentration (mg g^{-1} dry tea) Amperometric	Relative Error (%)
Yerba mate tea	2.15 ± 0.01*	2.13 ± 0.03	- 0.93
Black tea	2.71 ± 0.04	2.69 ± 0.02	- 0.74
Chamomile tea	1.85 ± 0.02	1.84 ± 0.03	- 0.55
Mint tea	0.15 ± 0.01	0.15 ± 0.01	0.00
Fennel tea	< LOQ	< LOQ	-
Green tea	< LOQ	< LOQ	-

* standard deviation of the mean with a confidence level of 95%, for n=3.

CONCLUSIONS

In conclusion, developing biosensors using cellophane paper immobilized with polyphenol oxidase enzymes from crude extracts of dwarf banana and eggplant peels, glutaraldehyde, and a Clark electrode proved to be a cost-effective alternative to Teflon® membrane electrodes for polyphenol detection in teas. The biosensors optimized enzyme concentration and pH value, exhibiting greater analytical performances, and the polyphenol levels found agreed with those obtained by the standard method with an acceptable error range (<1%). Despite the lower number of measurements for cellophane membranes compared to Teflon, the easy access and lower cost of cellophane paper allow for constructing several membranes, resulting in cost advantages and functioning as a disposable membrane. Therefore, the proposed method offers a promising approach to the detection of polyphenols in teas and has the potential to be extended to other applications.

Conflicts of interest

Authors declare no conflicts of interest.

Acknowledgements

The authors are grateful to São Paulo Research Foundation (FAPESP) [Grant number 2018/08782-1], “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) [Grant number 302839/2020-8] and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil” (CAPES) – Finance Code 001 for the financial support.

REFERENCES

(1) Fesko, K. Comparison of L-Threonine Aldolase Variants in the Aldol and Retro-Aldol Reactions. *Front Bioeng. Biotechnol.* **2019**, *7*, 119. https://doi.org/10.3389/fbioe.2019.00119

(2) Yan, Y.; Kim, S. H.; Zhang, F.; Piao, L.; Zhou, H. Rapid Macroscopic-Scale Assembly of Ag Nanowires at the Water/Air Interface. *J. Nanosci. Nanotechnol.* **2018**, *18* (3), 1995-2000. https://doi.org/10.1166/jnn.2018.14254

(3) Anastasiadis, V.; Koukouvinos, G.; Petrou, P. S.; Economou, A.; Dekker, J.; Harjanne, M.; Heimala, P.; Goustouridis, D.; Raptis, I.; Kakabakos, S. E. Multiplexed mycotoxins determination employing white light reflectance spectroscopy and silicon chips with silicon oxide areas of different thickness. *Biosens. Bioelectron.* **2020**, *153*, 112035. https://doi.org/10.1016/j.bios.2020.112035

(4) Kumar, V.; Sinha, A. K.; Uka, A.; Antonacci, A.; Scognamiglio, V.; Mazzaracchio, V.; Cinti, S.; Arduini, F. Multi-potential biomarkers for seafood quality assessment: Global wide implication for human health monitoring. *TrAC, Trends Anal. Chem.* **2020**, *132*. https://doi.org/10.1016/j.trac.2020.116056

(5) Chang, Y. F.; Wang, W. H.; Hong, Y. W.; Yuan, R. Y.; Chen, K. H.; Huang, Y. W.; Lu, P. L.; Chen, Y. H.; Chen, Y. A.; Su, L. C.; et al. Simple Strategy for Rapid and Sensitive Detection of Avian Influenza A H7N9 Virus Based on Intensity-Modulated SPR Biosensor and New Generated Antibody. *Anal. Chem.* **2018**, *90* (3), 1861-1869. https://doi.org/10.1021/acs.analchem.7b03934

(6) Pérez-Pérez, T.; Pereda-Reyes, I.; Correia, G. T.; Pozzi, E.; Kwong, W. H.; Oliva-Merencio, D.; Zaiat, M.; Montalvo, S.; Huiliñir, C. Performance of EGSB reactor using natural zeolite as support for treatment of synthetic swine wastewater. *J. Environ. Chem. Eng.* **2021**, *9* (1). https://doi.org/10.1016/j.jece.2020.104922

(7) Vieira, L. M.; Castro, C. F. S.; Dias, A. L. B.; Silva, A. R. Fenóis totais, atividade antioxidante e inibição da enzima tirosinase de extratos de Myracrodruon urundeuva Fr. All. (Anacardiaceae). *Rev. Bras. Plant. Med.* **2015**, *17* (4), 521-527. https://doi.org/10.1590/1983-084x/13_033

(8) Thomas, P. J.; Hellevang, J. O. A high response polyimide fiber optic sensor for distributed humidity measurements. *Sens. Actuators, B* **2018**, *270*, 417-423. https://doi.org/10.1016/j.snb.2018.05.011

(9) Gogalic, S.; Sauer, U.; Doppler, S.; Preininger, C. Investigating Colorimetric Protein Array Assay Schemes for Detection of Recurrence of Bladder Cancer. *Biosensors* **2018**, *8* (1). https://doi.org/10.3390/bios8010010

(10) Pooja, D.; Singh, L.; Thakur, A.; Kumar, P. Green synthesis of glowing carbon dots from *Carica papaya* waste pulp and their application as a label-freechemo probe for chromium detection in water. *Sens. Actuators, B* **2019**, *283*, 363-372. https://doi.org/10.1016/j.snb.2018.12.027

(11) Hong, S.; Kim, J. S.; Lee, J. U.; Lee, S. M.; Kim, J. H.; Ahn, J. Evaluation of Metal Absorber Materials for Beyond Extreme Ultraviolet Lithography. *J. Nanosci. Nanotechnol.* **2015**, *15* (11), 8652-8655. https://doi.org/10.1166/jnn.2015.11512

(12) Chae, S. C.; Hur, J.; Kim, I. T. Sb/Cu_2Sb-TiC-C Composite Anode for High-Performance Sodium-Ion Batteries. *J. Nanosci. Nanotechnol.* **2016**, *16* (2), 1890-1893. https://doi.org/10.1166/jnn.2016.11932

(13) Fatibello Filho, O.; Souza, M. G. d.; Vieira, I. d. C. Determinação espectrofotométrica por injeção em fluxo de compostos fenólicos em águas residuárias empregando peroxidase de abobrinha (Cucurbita pepo). *Ecletica Quim.* **2002**, *27*. https://doi.org/10.26850/1678-4618eqj.v27.1.2002.p51-66

(14) Signori, C. A.; Fatibello-Filho, O. Biossensor amperométrico para a determinação de fenóis usando um extrato bruto de inhame (Alocasia macrorhiza). *Quim. Nova* **1994**, *17* (1), 5. https://quimicanova.sbq.org.br/detalhe_artigo.asp?id=5471

(15) Oliveira, D. P. C. d.; Ribeiro, F. W. P.; Becker, H.; Lima-Neto, P.; Correia, A. N. An Electrochemical Biosensor Based on the Tyrosinase Enzyme for the Determination of Phenol in Wastewater. *Quim. Nova* **2015**. https://doi.org/10.5935/0100-4042.20150086

(16) Baudrit J, V. Recycling and Elimination of Wastes obtained from Agriculture by using Nanotechnology: Nanosensors. *International Journal of Biosensors & Bioelectronics* **2017**, *3* (5). https://doi.org/10.15406/ijbsbe.2017.03.00084

(17) Sousa, C. F. B. d.; Mota, L. T. M.; Valente, R. K. M.; Gomes, W. E.; Silva, V. C. d. S. Implementação de Biossensor Portátil Utilizando Tecnologia Wi-Fi para Detecção de Compostos Fenólicos em Ambiente Urbano. *Brazilian Journal of Development* **2020**, *6* (8), 59469-59480. https://doi.org/10.34117/bjdv6n8-387

(18) Qi, Z.; Dong, H.; Koenig, G. M. Electrochemical Characterization of Lithium-Ion Battery Cathode Materials with Aqueous Flowing Dispersions. *Electrochim. Acta* **2017**, *253*, 163-170. https://doi.org/10.1016/j.electacta.2017.09.031

(19) Tegou, A.; Papadimitriou, S.; Armyanov, S.; Valova, E.; Kokkinidis, G.; Sotiropoulos, S. Corrigendum to “Oxygen reduction at platinum- and gold-coated iron, cobalt, nickel and lead deposits on glassy carbon substrates” [J. Electroanal. Chem. 623(2008) 187]. *J. Electroanal. Chem.* **2009**, *625* (2). https://doi.org/10.1016/j.jelechem.2008.10.006

(20) Lee, H.; Chang, B.-Y.; Kwack, W.-S.; Jo, K.; Jeong, J.; Kwon, S.-H.; Yang, H. Dependence of the capacitance between an electrode and an electrolyte solution on the thickness of aluminum oxide layers deposited using atomic layer deposition. *J. Electroanal. Chem.* **2013**, *700*, 8-11. https://doi.org/10.1016/j.jelechem.2013.04.008
(21) Rudolph, S.; Schröder, U.; Bayanov, I. M. On-line controlled state of charge rebalancing in vanadium redox flow battery. *J. Electroanal. Chem.* **2013**, *703*, 29-37. https://doi.org/10.1016/j.jelechem.2013.05.011
(22) Swartz, H. M.; Mason, R. P.; Hogg, N.; Kalyanaraman, B.; Sarna, T.; Plonka, P. M.; Zareb, M.; Gutierrez, P. L.; Berliner, L. J. Free Radicals and Medicine. In: Eaton, S. R.; Eaton, G. R.; Berliner, L. J. (Eds.) *Biological Magnetic Resonance: Biomedical EPR, Part A: Free Radicals, Metals, Medicine, and Physiology*. Springer, New York, 2005. Vol. 23, Chapter 3, pp 25-74.
(23) Eremenko, A.; Makower, A.; Jin, W.; Ruger, P.; Scheller, F. Biosensor based on an enzyme modified electrode for highly-sensitive measurement of polyphenols. *Biosens. Bioelectron.* **1995**, *10* (8), 717-722. https://doi.org/10.1016/0956-5663(95)96962-x
(24) Mariano-Nasser, F. A. d. C.; Borges, C. V.; Ramos, J. A.; Nasser, M. D.; Lundgren, G. A.; Furlaneto, K. A.; Kovalski, T. R.; Vieites, R. L. Bioactive compounds and enzymatic activity in minimally processed eggplant packedunderactive modified atmosphere. *Semina: Ciências Agrárias* **2019**, *40* (1). https://doi.org/10.5433/1679-0359.2019v40n1p139
(25) Gregor, A.; Kostrzewska, E.; Godorowska, W. Determination of serum proteins in the presence of dextran by means of the Biuret reaction. *Infusionstherapie und klinische Ernährung* **1977**, *4* (1), 48-50. https://doi.org/10.1159/000219790
(26) Alves, I. A. B. d. S.; Miranda, H. M.; Barbosa, A. P.; Randau, K. P.; Soares, L. A. L. Desenvolvimento e validação de metodologia analítica por espectrofotometria no visível para quantificação de taninos totais na casca do caule de *Simarouba amara* Aubl. *Revista Árvore* **2015**, *39* (1), 37-47. https://doi.org/10.1590/0100-67622015000100004
(27) Kiralp, S.; Toppare, L.; Yagci, Y. Immobilization of polyphenol oxidase in conducting copolymers and determination of phenolic compounds in wines with enzyme electrodes. *Int. J. Biol. Macromol.* **2003**, *33* (1-3), 37-41. https://doi.org/10.1016/s0141-8130(03)00062-x
(28) Macholán, L.; Scháněl, L. Enzyme electrode with immobilized polyphenol oxidase for determination of phenolic substrates. *Collect. Czech. Chem. Commun.* **1977**, *42* (12), 3667-3675. https://doi.org/10.1135/cccc19773667
(29) Rosbash, D. O. Preparation of Folin-Ciocalteu's phenol reagent using 30% hydrogen peroxide. *Am. J. Clin. Pathol.* **1949**, *19* (3), 297. https://doi.org/10.1093/ajcp/19.3_ts.297