ACRYLAMIDE DETECTION USING ELECTROCHEMICAL ANALYSIS. DETERMINATION WITH BIOCHAR MODIFIED CARBON PASTE ELECTRODE (MCPE)

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Abstract. The coffee is one of the most consumed industrial products nowadays, it is present in various environments, in various forms and has been consumed by individuals of different age groups, its bean is found in large quantities in Brazil being the fruit of the coffee tree, its industrialization process has several stages, being the roasting the stage of interest to this work.
At this stage, the indirect formation of acrylamide occurs through the Milliard process, where substances present in coffee, such as asparagine and saccharides, are heated at temperatures above 120°C, forming acrylamide.
In 1994, the International Agency for Research on Cancer (IARC) classified Acrylamide (CAS No. 79-06-1) as a possible human carcinogen (Group 2A), since then studies have been made to understand its formation in food and the effects caused in humans.
This work had as main objective to determine acrylamide in coffee samples, however, the use of electroanalytical methods such as cyclic voltammetry (CV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV) were not efficient in the research, one of the hypotheses discussed for such result came from the analysis of the high polarity and low molecular mass of the compound, methods such as Gas Chromatography (GC) or Liquid Chromatography (LC) and Mass Spectrometry (MS) are the most common and appropriate for the identification and determination of acrylamide.
The results of the identification tests of acrylamide in solution of tetrabutylammonium bromide (TBAB)[0,1 mol L-1], using carbon paste electrodes modified with biochar (MCPE), are presented, using CV, DPV and SWV. Analysing the results obtained resulted in the hypothesis that the biochar present in the MCPE absorbed the acrylamide present in solution, hindering the determination of the substance.

Keywords. Acrylamide, Biochar, Modified Carbon Paste Electrode, Coffee, Voltammetry.

1. Introduction

Coffee is one of the most consumed industrial products nowadays, it is present in various environments, in various forms and has been consumed by individuals of different age groups, its bean is found in large quantities in Brazil being the fruit of the coffee tree.

Currently Brazil is the largest exporter of soluble coffee in the world, exporting 0.32 million bags in March 2023 **[1]** and has been classified for years by the IOC (International Coffee Organization) as one of the largest producers and exporters of coffee in the world, studies conducted by the IOC indicate that coffee consumption increased during the years 2021-2023 (years of the Covid-19 pandemic) **[1]** by at least 4.0% (2021/22).

The process of industrialization of coffee is composed of several stages, being the collection and selection of the grains, processing, roasting, grinding, packaging and labelling of the grains and marketing. For this work, the stage of interest will be the roasting.

The step analysed in this research is the roasting, which according to ABIC (Brazilian Association of Coffee Industry) consists in the transformation process of the green bean into a roasted bean, which will be used for the manufacture of soluble and liquid coffee; this heating causes endothermic and exothermic reactions that start the process of mass transfer of the bean.

The main reaction present in the heating of foods rich in reducing sugars and amino acids is the Maillard reaction, which in this work was analysed

as the formation reaction of acrylamide (figure 1)

Acrylamide is a chemical substance (C_3H_5NO), solid at room temperature, soluble in water, organic solvents such as ethanol, acetone, ethyl acetate and benzene and has a low melting point (84.5°C). Its formation occurs indirectly in heating reactions at temperatures above 120° C. In 1994, the International Agency for Research on Cancer (IARC) classified Acrylamide (CAS No. 79-06-1) as a possible human carcinogen (Group 2A).

Research suggests that acrylamide is neurotoxic to human health (Food and Agriculture Organization/World Health Organization, 2002) and studies have been conducted to understand its contribution to genetic modification and propensity to develop cancer in humans.

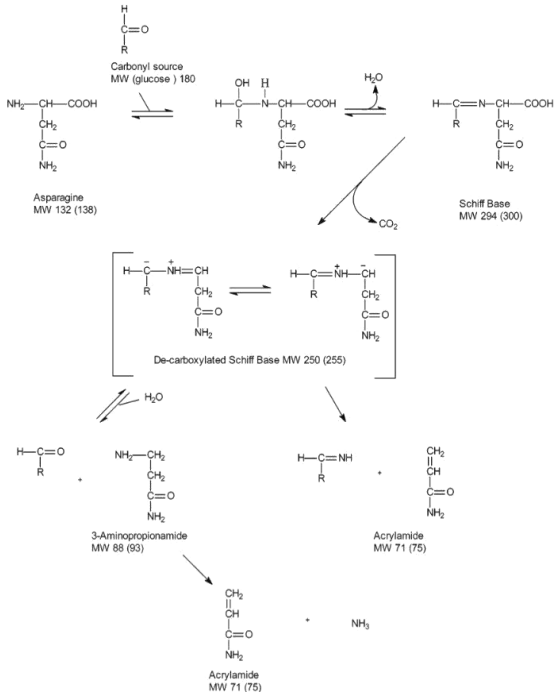

Figure 1. Mechanism of the formation reaction of acrylamide by the Maillard process **[2]**

2. Discussed Topics

2.1 Acrylamide

The determination of acrylamide has been made by gas chromatography (GC), liquid chromatography (LC), square wave voltammetry (SWV), infrared spectrometry, among other methods.

This work aimed to investigate the determination of acrylamide using carbon paste electrodes modified with biochar.

Acrylamide is a chemical substance (C_3H_5NO), solid at room temperature, soluble in water, organic solvents such as ethanol, acetone, ethyl acetate and benzene, heptane and methanol; it has a low melting point (84.5°C). Its formation can occur indirectly from the heating of foods rich in reducing sugars and amino acids at temperatures above 120°C.

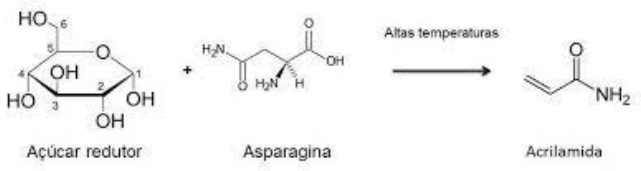

Figure 2. Simplified reaction of the formation of acrylamide by the Maillard process. **[4]**

It has a high carcinogenic index, according to the IOC, it has been classified as possibly carcinogenic, and can cause changes in sperm morphology, reproductive system, by interacting with DNA, inducing genetic mutations and causing neurological toxicity. **[3]**

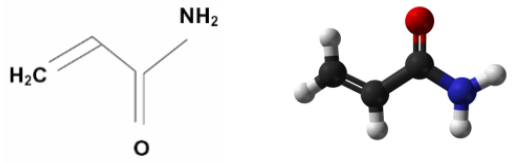

Figure 3. Structural formula of acrylamide **[4]**

2.2 Biochar

Biochar is a carbonaceous solid formed from the pyrolysis reaction, a process of thermal decomposition of organic matter. It is mostly composed of amorphous carbons, i.e. carbons that do not have a well-defined structural arrangement in crystalline form.

The biochar is a material of low production cost and very sustainable, since its production can be made from a variety of organic matter, biomass of animal or plant origin and agricultural or industrial waste, in this work, the biochar used for the construction of the carbon paste electrode was made from sugar cane bagasse.

The biochar has been widely studied by researchers due to its physical and chemical properties. Its amorphous structure and porous surface allows the analyte studied to be absorbed more efficiently because of the increase of the surface area and the electrical conductivity of the electrode.

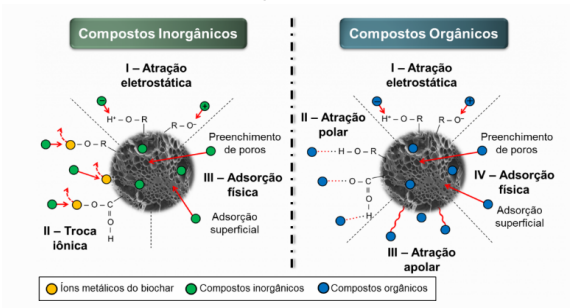

Figure 3. Interaction of biochar with organic and inorganic compounds. (I - electrostatic attraction; II-

ionic exchange; III- physical adsorption) **[5]**

2.3 MCPE with Biochar

The construction of the modified carbon paste electrode (MCPE) was made from a binder (paraffin), the modifier (biochar) and lollipop stick

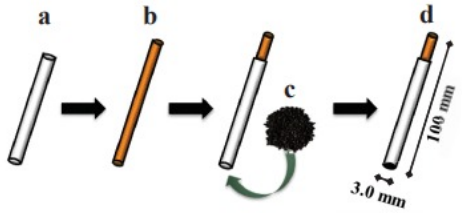

Figure 4. Construction of carbon paste electrode **[6]**

The paste was made from a mixture of 25% (m/m) binder (paraffin), 60% graphite and 15% modifier (biochar).

An important point to be highlighted is that initially the electrode was constructed using nujol mineral oil as the electrode binder, however, the potentials applied (UP: 1,0V; LP: -1.0V) in the tests damaged the carbon paste and the electrode was destroyed; After some tests performed with other solutions of ascorbic acid and PBS, to confirm the quality of the binder, the mineral oil nujol was replaced by paraffin, and the following steps of the experiment were performed without major problems.

3. Methodology

The methodology applied to experimental tests was carried out in the Laboratory of Electrochemical Sensors (LabSense), provided by professors of the Department of chemistry of UFPR, Luiz Humberto Marcolino Junior and Marcio Fernando Bergamini.

During the process of the research the literature search for articles about cyclic voltammetry, biochar, assembly of carbon paste electrodes and acrylamide was made followed by literature search for the construction of the electrodes, the preparation of carbon paste [(1)], window tests with TBAB solution [0,1 mol L-1], application of CV, WSV and DPV for the detection of acrylamide in solution.

The experiment was performed to monitor cathodic reduction of acrylamide, a reaction proposed by Niaz et al. (2008) **[7]**

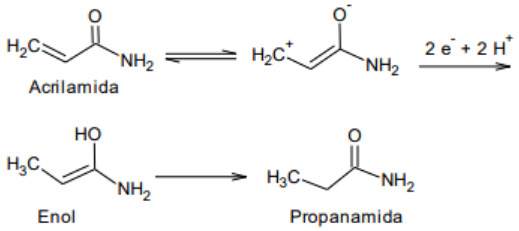

Figure 5. Reaction mechanism of acrylamide reduction in aqueous media proposed by Niaz (using a Hg electrode). **[7]**

The main topics investigated were the absorption properties of biochar in relation to acrylamide, and the methods already performed for the detection of acrylamide in solution and the development of a new method of electrochemical analysis using a carbon paste electrode, in view of the fact that acrylamide has already been detected using vitreous carbon electrodes **[7]**.

4. Results

After the research and the experiments carried out, the results obtained through cyclic voltammetry were not satisfactory, considering that the objective of the study was to perform the determination of acrylamide in the coffee commercialised in Brazil. However, the first stage of the research, the realisation of the detection of acrylamide in TBAB solution [0,1 mol L-1] was not successful, the graphs obtained from the CV, SWV and DPV did not show signs of the analyte in the solution.
Thus, the subsequent processes for the detection and determination of acrylamide in coffee samples were unfortunately not performed.
It could be observed that when acrylamide was added in the solution, the system behaved in a capacitive manner, instead of obtaining a clear signal from the sample, the system showed high background currents.This phenomenon was observed with each addition and each voltammetric cycle performed (Figure 6).

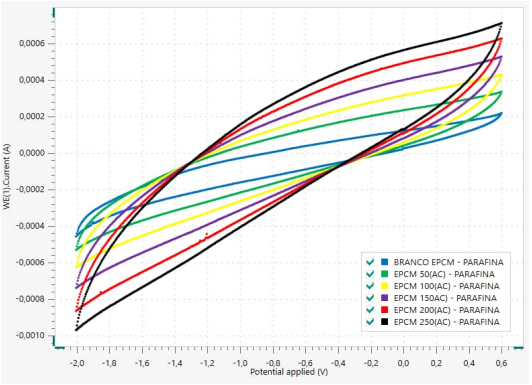

Figure 6. Cyclic voltammetry of acrylamide in TBAB solution (tetrabutylammonium bromide) [0.1 mol L^{-1}], scan rate (0.1 V/s), AC = acrylamide. Blue line (white test); Green line (50 µl-AC); Yellow line (100 µl-AC); Purple line (150 µl-AC); Red line (200 µl-AC); Black line (250 µl-AC).[AC] = 0,001 mol/L .

After the analysis of the results from cyclic voltammetry, Square Wave Voltammetry (SWV) and Differential Pulse Voltammetry (DPV) were performed to obtain a more detailed picture of the system. However, as can be seen in figures 7 and 8, no signal from the analyte in solution was observed.

All additions were started with 50µl of acrylamide and finished with 250µl of acrylamide in solution.

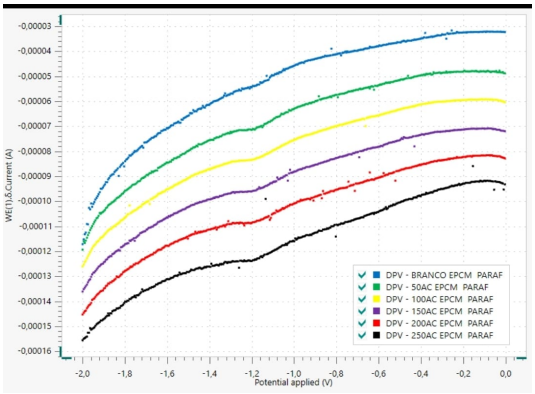

Figure 7. DPV of acrylamide in TBAB solution[0,1 mol L-1] using MCPE, scan rate (0,1 V/s), AC = acrylamide. Blue line (white test); green line (50 µl-AC); yellow line (100 µl-AC); purple line (150 µl-AC); red line (200 µl-AC); black line (250 µl-AC).

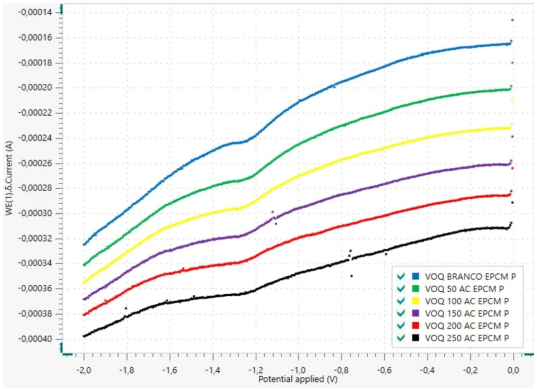

Figure 8. SWV of acrylamide in TBAB solution[0,1 mol L-1] using MCPE, scan rate (0,1 V/s), AC = acrylamide. Blue line (white test); green line (50 µl-AC); yellow line (100 µl-AC); purple line (150 µl-AC); red line (200 µl-AC); black line (250 µl-AC).

In a last attempt to detect acrylamide in solution, a glassy carbon electrode was used, which was polished with alumina powder to obtain a better contact surface, followed by voltammetric tests and again no signal from the sample in solution was found.

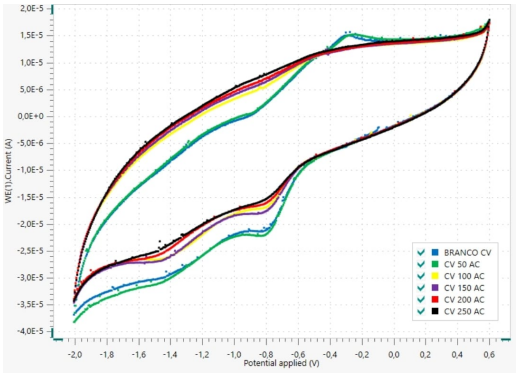

Figure 9. Cyclic voltammetry of acrylamide in TBAB solution[0,1 mol L-1], using glassy carbon electrode, scan rate (0,1V/s), AC = acrylamide. Blue line (white test); Green line (50 µl-AC); Yellow line (100 µl-AC); Purple line (150 µl-AC); Red line (200 µl-AC); Black line (250 µl-AC).

5. Discussion and Hypothesis

From the results obtained, some hypotheses were suggested about what may have occurred during the experiments.

We began the studies with the construction of the carbon electrode using the nujol as binder, however, we did not obtain positive results as to its resistance see paragraph 2.3, we performed the replacement of the binder by paraffin and continued with the tests.

The results of the cyclic voltammetry were analysed, however, the results showed that with each addition of acrylamide only the background current increased. It can be explained by porous characteristic of the biochar surface, which in contact with acrylamide may absorbed the analyte in solution (it was not possible to predict a chemical reaction for the process), making it impossible to detect the acrylamide by the voltammetric method used.

Another possibility is to use the voltammetric method used in the experiment and the medium (TBAB solution [0.1 mol L^{-1}]) in which the research was conducted, which was taken as the basis of other studies carried out for the determination of acrylamide using the glassy carbon electrode modified with Hg film, in KCl solution [0.1 mol L-1] **[7]**.

6. Conclusion

The analysis method chosen for this experiment was not efficient making it impossible to meet the objective of the work, the use of the carbon paste electrode modified with biochar did not present significant and relevant results for determination of biochar..
The probable absorption of acrylamide by the modifier made it impossible to detect the analyte in solution. Moreover, it can be added that nujol has limitations regarding its use as a binder in a MCPE, a result observed and significant for future research.

7. Acknowledgement

I would like to thank all the people who supported me during this process of research and studies, there were many hours dedicated and many people were with me in difficult moments when I doubted my abilities to continue in the program.
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Finally, I would like to thank God, who guided me and allowed this work to be carried out.

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