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Activated Carbon Paste Electrode for Voltammetric Determination of Butylated Hydroxyanisole

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ABSTRACT

Butylated Hydroxyanisole (BHA) is an antioxidant with phenolic groups commonly added to food materials as an oxidative deterrent. According to The Acceptable Daily Intake (ADI), the permissible human consumption limit for BHA is very low. Therefore, having a sensitive and simple analytical method for BHA determination is important. In this study, we developed an activated carbon paste electrode (aCPE) for the voltammetric determination of BHA in food samples. Carbon paste electrode (CPE) was activated in 1 M H_2SO_4 solution in two steps: 10 cycles of cyclic voltammetry followed by immersion in 1 M H_2SO_4 solution for 30 seconds. The aCPE electrode had been used for BHA determination in food samples and the results were in good agreement with those of HPLC.

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INTRODUCTION

Butylated Hydroxyanisole (BHA) is an antioxidant commonly added to food materials. The presence of BHA in food is intended to eliminate rancid odor, ensuring the quality of the food remains preserved. Antioxidants are important substances commonly added to food items in the presence of free radicals (Tomášková et al., 2014). Therefore, the role of antioxidants is to inhibit free radicals and transform them into stable products through redox reactions. The Acceptable Daily Intake (ADI) value for BHA consumption is 0 - 0.5 mg/kg. Due to its very low threshold value, it is necessary to control and monitor the daily consumption levels. (Jakubczyk and Michalkiewicz., 2018). Several methods have been used for BHA determination such as spectrophotometry, High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Micellar Electrokinetic Capillary Chromatography (MECC) (Jakubczyk and Michalkiewicz., 2018). However, each instrument has its advantages and numerous drawbacks, such as challenging sample preparation, relatively expensive equipment, lengthy procedures, and even the need for prior analyte extraction (Liu et al., 2005).

Electroanalytical techniques are commonly employed to ascertain the content of electroactive species in sample solutions. Because BHA is electroactive, its determination can be conducted using the voltammetric method. The voltammetric method has several advantages because it has a relatively fast analysis time and low analysis costs. It also produces negligible waste and mostly uses water as a solvent. The size of the instruments is small and nowadays battery-operated instruments are available so they can be used for fieldwork.

Several electrodes have been used in voltammetric measurements such as gold, platinum, glassy carbon, and carbon paste electrode (CPE). The electrochemical behavior of CPE is associated with its composition and surface structure. However, unmodified carbon paste electrodes exhibit low activity for certain measurements, indicating the presence of a less favorable electrode surface. Various treatments have been carried out to enhance the surface performance of the electrode. In this study, we activated CPE in 1 M H_2SO_4 solution with the cyclic voltammetry (CV) technique and used the electrode to determine BHA in the food sample.

LITERATURE REVIEW

Antioxidant

Antioxidant additives in food aim to enhance the characteristics of foodstuffs (storage parameters, taste and color, nutritional value, and health effects). Synthetic antioxidants, especially those with phenolic properties, are typically used for products containing oils and fats to prevent oxidation processes caused by lipid peroxidation. The most common antioxidants in food are tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) (Ziyatdinova et al., 2020). Antioxidants are added to food at low concentrations compared to oxidizable substrates (Thomas et al., 2015). In general, they prevent or counteract the oxidation of these substrates. Antioxidants are also of interest in the fields of biochemistry and

health because they greatly assist the body in protecting itself from damage caused by reactive oxygen species (ROS), as well as nitrogen species (RNS) and chlorine species (RCS). The stages of the autooxidation reaction are as follows:
Initiation : $RH \rightarrow R\bullet$
Propagation : $R\bullet + O_2 \rightarrow ROO\bullet$
$ROO\bullet + RH \rightarrow ROOR$
Termination : $R\bullet + R\bullet \rightarrow R - R$
$ROO\bullet + R\bullet \rightarrow ROOR$
$ROO\bullet + ROO\bullet \rightarrow$ Non-radical products

The initiation process generates free radicals from the substrate. Antioxidants are components that prevent the auto-oxidation of oils and fats by giving their hydrogen to free radicals formed in the initiation and propagation stages of autoxidation by the following reactions (AH is an antioxidant molecule):
$ROO\bullet + AH \rightarrow ROOH + A\bullet$
$R\bullet + AH \rightarrow RH + A\bullet$
$RO\bullet + AH \rightarrow ROH + A\bullet$
$ROO\bullet + A\bullet \rightarrow ROOA$
$RO\bullet + A\bullet \rightarrow ROA$
$A\bullet + A\bullet \rightarrow A - A$ (Taghvaei & Jafari, 2015).

Butylated Hydroxyanisole

BHA has antioxidant capabilities, as evidenced by its resistance to various processing stages and stability in the final products, particularly in food products containing animal fats but relatively less effective in foods containing plant oils. BHA is an antioxidant that combines two isomeric phenolic compounds, namely 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole. These compounds are insoluble in water but soluble in methanol and ethanol. BHA is frequently used as an additive in food and cosmetics due to its antioxidant properties. The active part of BHA acting as an antioxidant is its conjugated aromatic ring, which serves as a stabilizer for free radicals, thereby preventing further free radical reactions. Synthetic antioxidants like BHA are known to exhibit higher antioxidant activity compared to vitamin E.

Butylated Hydroxyanisole (BHA) is a synthetic antioxidant that typically contains a phenolic group and is commonly used as a preservative in cosmetics, food, biodiesel, and various pharmaceutical applications (Manoranjitham and Narayanan., 2021). BHA significantly contributes to enhancing product stability over an extended period by preventing oxidation through the donation of protons to free radicals. The resulting free radicals are neutralized by the conjugated aromatic ring present in the structure of BHA. The oxidation reaction of BHA on the surface carbon paste electrode is shown in Figure 1.

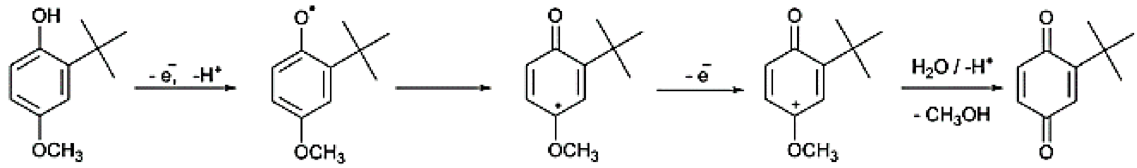

Figure 1. Electrochemical oxidation of BHA (Ziyatdinova et al., 2015).

Antioxidants with phenolic groups can exhibit carcinogenic effects if they exceed the recommended dosage. Therefore, BHA is a substance that needs to be strictly controlled. The Food and Drug Administration (FDA) has established the permissible level of BHA consumption, which is 100-200 mg/kg in oils or lipids. Due to the low recommended maximum limit, it is necessary to develop an accurate method for its determination.

Voltammetry

The term "voltammetry" is coined from abbreviations referring to the units, namely volt (V) and ampere (A), plus the suffix "metry" with the meaning of measuring. Thus, volt+am+metry = voltammetry. The scheme of the voltammetric cell is as follows:

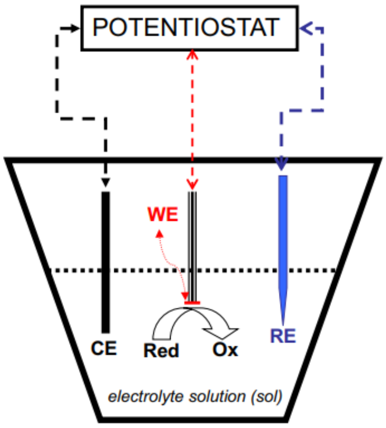

Figure 2. Scheme of voltammetric cell (Mirceski et al., 2018).

Voltammetry is an electrochemical measurement method where the measured current is a function of the applied potential. In voltammetry, the relationship between the measured current and the applied potential is observed during an electrochemical process. Therefore, measurements using voltammetry are based on the oxidation or reduction reactions of the analyzed compounds. The redox reaction is as follows:

$$\mathrm{Red_{sol}} \rightleftarrows \mathrm{Ox_{sol}} + ne^-$$

Voltammetry is an effective method developed for the analysis of additive substances like BHA. This electrometric-based method has numerous advantages, such as high sensitivity, rapid response, simple, cost-effective, and environmentally friendly. Recent advancements in this method focus on improving the performance of carbon paste electrodes (CPE) as the working electrode, particularly in enhancing sensitivity. The Square Wave Voltammetry (SWV) technique has high sensitivity in electroanalysis and is also the most stable technique in studying electrode kinetics and reaction mechanisms of redox systems (Batchelor-Mcauley et al., 2015).

Square Wave Voltammetry

Square wave voltammetry is one of the voltammetric measurement techniques that exhibits excellent sensitivity. Generally, the SWV technique is similar to Differential Pulse Voltammetry (DPV) technique. In SWV measurements, there are cathodic pulses and anodic pulses. During the cathodic pulse, the analyte undergoes a reduction process on the electrode surface, while during the anodic pulse, the newly reduced analyte is oxidized again.

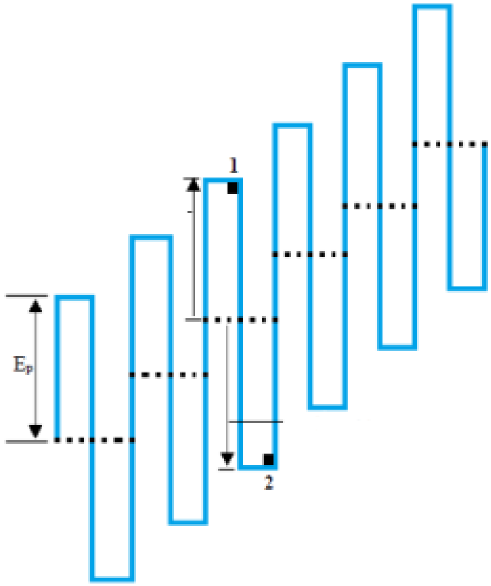

Figure 3. Square Wave Voltammetry (Mirceski et al., 2018).

In Figure 3, points 1 and 2 are observed. Electrons will flow from the electrode to the analyte at point 1 (reduction) and from the analyte to the electrode at point 2 (oxidation). Since these two currents have opposite signs due to the opposite direction of electron flow, the difference between these two currents will be larger compared to their values. When the difference in current is plotted, it is found that the voltammogram resulting from the SWV technique is a derivative curve of the polarogram. The signal obtained in SWV measurements tends to be larger or increased. The advantage of the SWV technique is that measurements with SWV are faster compared to other voltammetric techniques (Mirceski et al., 2018).

METHODOLOGY

Apparatus

All voltammetric measurements were conducted using an eDAQ potentiostat with a traditional three-electrode system: a CPE as a working electrode, a Pt-wire as an auxiliary electrode, and an Ag/AgCl as a reference electrode. HPLC measurements were conducted with an Agilent 1260 infinity manual injector.

Chemical reagents

BHA (Sigma Aldrich), graphite (Sigma Aldrich), paraffin liquid (Sigma Aldrich), glacial acetic acid (Sigma Aldrich), ethanol (Merck), KNO_3 (Merck), H_2SO_4 (Merck), methanol (Merck), acetonitrile (Merck), $K_3[Fe(CN)_6]$ (Merck), $K_4[Fe(CN)_6]$ (Merck), NaCl (Merck), NaH_2PO_4 (Merck), Na_2HPO_4 (Merck), deionized distilled water.

Preparation of activated carbon paste electrode

The carbon paste electrode is made by adding a mixture of graphite and paraffin liquid in a ratio of 7:3. Afterward, the paraffin liquid is heated to 80^0C and stirred until homogeneous. The carbon paste is then filled into the electrode body. The activation process is carried out in two steps: a 10 potential cycle (0.0 mV – 1200 mV vs Ag/AgCl) using cyclic voltammetry in 1 M H_2SO_4 solution and followed by immersing it in the same solution for 30 seconds.

Treatment of commercial food samples

A similar procedure proposed previously (Medeiros et al., 2010) was followed for the determination of BHA in commercial cheese. In 60 grams sample, 30 mL of ethanol was added, and the mixture was centrifuged for 30 minutes at 6000 rpm to extract the BHA. The BHA concentration in ethanol extract was determined voltammetrically and with the HPLC method. HPLC measurements were conducted using a C18 column with a mixture of 1% glacial acetic acid: methanol: acetonitrile (4:3:3 v/v) as mobile phase and a UV detector (280 nm).

RESEARCH RESULT

Activation of carbon paste electrode

Figure 4 shows electrode activation 1 M H_2SO_4 solution. The surface of the electrode was activated using 1 M H_2SO_4 solution in two steps: 10 cycles of cyclic voltammetry followed by immersion in 1 M H_2SO_4 solution for 30 seconds (Hernández-Santos et al., 2005).

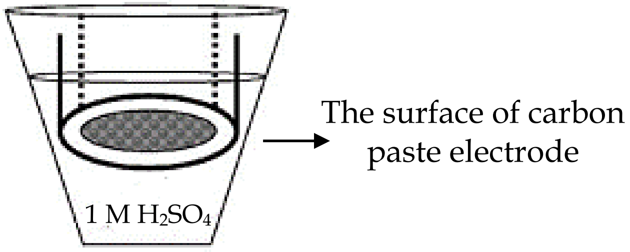

Figure 4. Activation of carbon paste electrode

Figure 5 shows 10 cycles of cyclic voltammogram (CV) of 1 M H_2SO_4 solution at a potential range of 0.0 mV – 1200 mV with a scan rate of 100 mV/s during the activation of CPE. An oxidation at 0.70 V decreases as the number of cycles increases. On the other hand, a reduction peak at 0.55 V increases as the number of cycles increases. After the potential cycles, the electrode was immersed in the same solution for 30 seconds before being used.

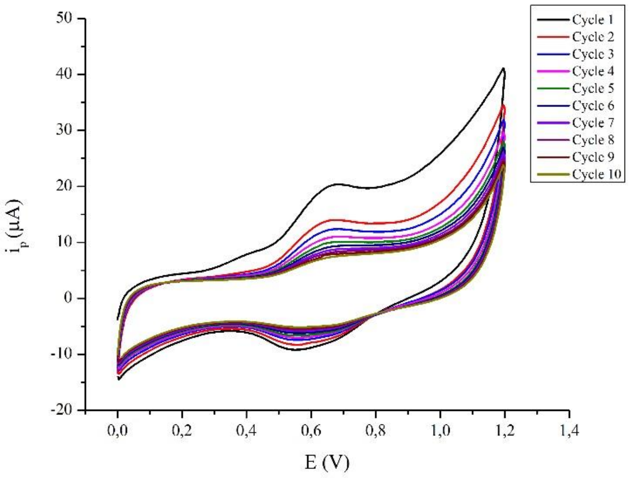

Figure 5. Ten cycles of CV of 1 M H_2SO_4 solution at a potential range of 0.0 mV – 1200 mV with a scan rate of 100 mV/s

Electrode evaluation

The performance of CPE and aCPE was compared by measuring 10 mM BHA in phosphate buffer 0.1 M (pH 7). The voltammograms are shown in Figure 6. The figure shows that the oxidation peak of BHA obtained with aCPE is significantly higher than that of CPE.

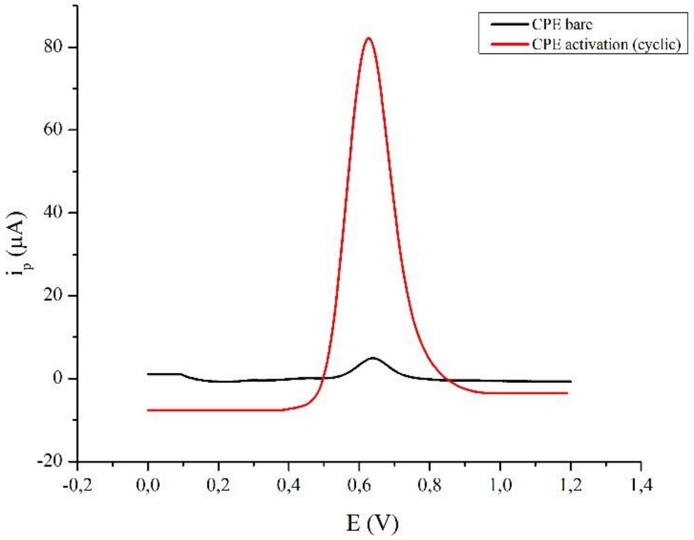

Figure 6. Square Wave Voltammogram oxidation peaks of 10 mM BHA in phosphate buffer 0.1 M (pH 7) were obtained using bare CPE and aCPE

Measurement of the sample

The extraction procedure was carried out with 1% glacial acetic acid: methanol: acetonitrile (4:3:3 v/v). The analysis was conducted by High

Performance Liquid Chromatography (HPLC), using UV detection at 280 nm for the analysis of BHA. The sample was analyzed with at least three injection sn. The peak of each antioxidant in the samples was identified by comparing the retention time to that of a standard. The results from the standards obtained linear equation. The obtained linear equation will be used to determine the concentration of the cheese sample (mM or mg/kg). For voltammetry determine BHA of the cheese sample using supporting electrolyte phosphate buffer 0.1 mM (pH 7). A potential range of 0.0 mV – 1200 mV with a scan rate of 100 mV/s and frequency of 10 Hz.

The accuracy of voltammetric determination of BHA with aCPE was conducted by comparing voltammetric results with those obtained with the HPLC method. The result of both methods for BHA content in the cheese sample is shown in Table 1. The equation for calculating %recorvery is formulated as follows:

$$\%\text{Recorvery} = \frac{\bar{x}}{x_{\text{ref}}} \text{ x } 100\%$$

Table 1. Measurement of BHA in cheese samples using HPLC and voltammetry

Sample	Voltammetry		HPLC	
	[BHA] (mM)	[BHA] (mg/kg)	[BHA] (mM)	[BHA] (mg/kg)
Cheese	0.23	0.22	20.81	20.56

The results obtained with both methods are similar to each other. Assuming that the HPLC results in a true value, the percent recovery of voltammetric results is 101.2 %, showing a good accuracy of voltammetric results.

DISCUSSION

Activation of CPE reduces the oxidation peak and increases the reduction peak indicating that the oxidation product had been reduced to activate the surface of the electrode. Activating the CPE surface increases BHA oxidation peak significantly from 5.50 μA to 87.20 μA showing a significant increase of electron transfer kinetics of BHA oxidation.

The reliability of aCPE for BHA determination was tested in the real sample and the results were compared to those obtained with the HPLC method. BHA content in cheese samples obtained with voltammetry and HPLC method were 0.23±0.01 and 0.22±0.03, respectively. The results show that the voltammetry method for BHA determination was as accurate as the HPLC method.

CONCLUSIONS AND RECOMMENDATIONS

CPE can be activated with cyclic voltammetry technique in 1 M H_2SO_4 solution to increase the electron transfer kinetics of BHA oxidation. The activated CPE can be used for voltammetric determination of BHA in real samples with good accuracy.

ADVANCED RESEARCH

Further optimization is necessary to achieve maximum sensitivity and selectivity, such as determining pH measurement, activation cycles, and voltammetry measurement techniques.

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