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Electrochemical Study of Auramine O at Glassy Carbon Electrode and Its Determination in Food by Differential Pulse Adsorptive Stripping Voltammetry

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Abstract- A simple, nontoxic, rapid and sensitive method to determine of auramine O in food using glassy carbon electrode was investigated. The cyclic, differential pulse adsorptive stripping voltammetry methods was used to study the electrochemical behavior of auramine O on the glassy carbon electrode in Britton-Robinson buffer. The oxidation peak of the auramine O was irreversible, adsorptive on the surface electrode. Using differential pulse adsorptive stripping voltammetry to determine auramine O with optimal conditions were Britton - Robinson buffer pH 10, adsorption potential 0 V, adsorption time 90 s, pulse amplitude 50 mV, scan rate 25 mV s^{-1}. This process could be used to determination auramine O concentration in the range from 1.0×10^{-8} mol L^{-1} to 20.0×10^{-8} mol L^{-1} with a detection limit of 2.7×10^{-9} mol L^{-1}, quantitation limit 9.0×10^{-9} mol L^{-1}. We successfully applied the procedure to determine auramine O in spiked chicken meat with mean recovery 90.2±2.1%. The detection limit and the limit of quantification of the auramine O in spiked chicken meat was found to be 0.16 µg g^{-1} and 0.53 µg g^{-1}, respectively.

Keywords- Auramine O, Glassy carbon electrode, Differential pulse adsorptive stripping voltammetry

1. INTRODUCTION

Auramine O (AO), bis[4-(dimethylamino) phenyl] methaniminium chloride (Figure 1), is the synthetic food color, very toxicity so it is unauthorized food additives in Japan, the EU, and the United Stated. The AO is very toxic to humans. It can cause carcinogenic, so the International Agency of Research on Cancer classified AO into Group 2B [1,2]. However, the AO have been detected in various foods such as chicken meat, chili sauce, marinated bamboo shoots, feed, fermented vegetable samples. The use of AO has been reported in several developing countries such as Philipines, India, Argentina, Malaysia, China and Vietnam [3,4].

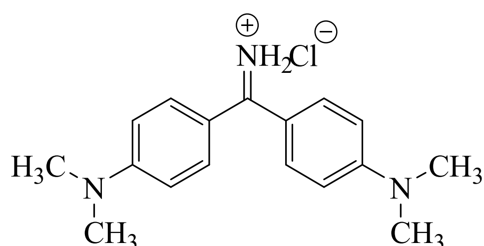


Fig. 1. Chemical structure of AO

According to the literature review, several methods such as spectrophotometry [5], liquid chromatography [4,6], liquid chromatography-tandem mass spectrometry [7], fluorescence [8,9], and terahertz spectroscopy [10] were reported for the determination of AO. Most of the methods are either expensive or and complicated because of time consuming procedure like preconcentration or multisolvent extraction and the high requirements of skilled technicians. So, we have chosen the voltammetry method which had highly sensitive, selective, easy to use, low cost and simple to determine AO in food. Specially, the adsorptive stripping voltammetry is an important analytical technique for determination of a wide range of electroactive organic compound. The AO was adsorbed on to the surface of the working electrode, especially glassy carbon electrode was non-toxic, wide electrochemical window. To the best of our knowledge, it is the first publication mentioning the adsorptive stripping voltammetry as the main analysis method to determine AO in food.

Our object article was to develop a simple, rapid and sensitive differential pulse adsorptive stripping voltammetric method (DPAdSV) on a glassy carbon electrode (GCE) for the determination of AO in food. This work focused on investigating clearly the detailed electrochemical behavior and oxidation mechanism of AO at GCE using cyclic voltammetry (CV) and DPAdSV. To obtain high sensitivity of the method, experiment conditions such as supporting electrolyte, pH, adsorption potential, adsorption time, scan rate, pulse amplitude were studied and optimized. The studies of method validation were performed on the aspects of stability, linearity, limit of detection, limit of quantitation, repeatability, precision, accuracy

and recovery. The developed voltammetric method was applied to determine of AO in food samples.

2. EXPERIMENTAL

2.1. Apparatus

The μAutolab type III (Netherlands), interfaced to the electrode assembly 663-VA (Metrohm, Switzerland), and controlled by software 757 VA computrace was used to research for determination auramine O in food samples. The electrochemical measurements were carried out in voltammetric cell containing of three electrodes with a glassy carbon working electrode. The reference electrode was an Ag/AgCl/KCl. A glassy carbon rod was used as counter electrode. A stirring rod provided the convective transport during preconcentration step. The dissolved oxygen was removed from the analyzed solution by passing high-purity nitrogen through the cell at least 5 mins before measurement performance.

2.2. Chemicals and reagents

AO (85% HPLC grade) was obtained from USA. All chemicals for the buffer preparations were at analytical reagent grade (Merck).

2.3. Standard solutions

A stock solution of 1.0×10^{-3} mol L^{-1} AO was prepared by dissolving a known, exactly amount of AO with double-distilled water. The stock solution was stored at 4 °C in the dark bottle. Standard solutions were diluted daily from the stock solution with double-distilled water. Working solution contained desired amount of AO in 10 mL buffer solution.

The Britton-Robinson (BR) buffers of pH 7-11 were prepared from a mixture of 0.04 mol L^{-1} acetic, orthophosphoric, boric acids (A solution) and 0.2 mol L^{-1} sodium hydroxide solution (B solution). To obtain required pH, we used pH meter to adjust pH of A solution by adding B solution. The BR buffers were used as supporting electrolytes.

2.4. Sample preparations from chicken meat

Firstly, chicken meat was finely ground until obtaining a well homogeneous mixture. 1 g chicken prepared samples were accurately weighted and poured in 4 mL of 50% etanol, shaken for 10 min and centrifuged at 6000 rpm for 15 mins to obtain the solution. The residual precipitates were treated samely two more times. All the solutions were collected into one flask. The solution, then, was soaked in hot water and filtered using a 0.45 μm filter paper. 3.0 mL of the filtrated solution, 10 mL BR buffer at pH 10.0 were added and diluted to exact 25.0 mL of volumetric flask by double-distilled water to obtain the working solution. The solution was

then poured to a voltammetric cell for the differential pulse measurement. AO concentration in chicken meat was determined by a standard addition method.

2.5. Analytical stripping procedure

We did the same preparation in section 2.4 for all working solutions with either the solution from chicken meat or variable concentrations of standard AO for the electrolysis cell. The cell was purged by a pure nitrogen stream for 5 min to remove all oxygen from the solution before recording the voltammograms. To preconcentrate of the AO on the electrode surface, a selected adsorption potential E_{ad}=0 V (*vs*. Ag/AgCl/KCl_s) was applied to the GCE for adsorption time t_{ad}=90 s. The solution was stirred during preconcentration time. After that the stirring rod was stopped for 5 s to allow the solution to become quiescent and working electrode surface to be uniformly distributed. Then the differential pulse technique with scanning potential towards the positive direction was applied to record voltammograms.

Cyclic voltammetric measurements worked in arrange of potential from 0 V to 1.2 V with different scan rates to study the electrochemical behaviors.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetry

The cyclic voltammetry was used to research the electrochemical behaviors of AO at glassy carbon electrode. The cyclic voltammogram of AO was showed in figure 2. To investigate the adsorptive characteristics of AO on the glassy carbon electrode surface, we recorded the cyclic voltammogram in two cases with conditions: 10^{-6} mol L^{-1} auramine O in BR buffer pH = 9.0 with non-preconcentration (curve 2) and preconcentration (curve 3) at the same adsorption potential of 0 V.

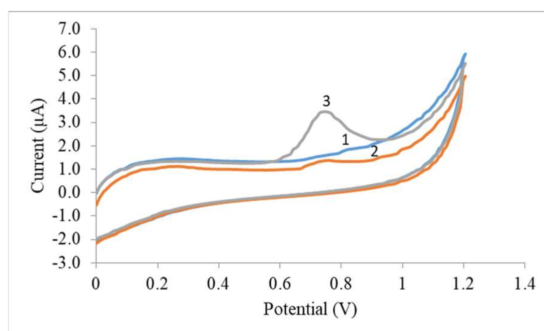


Fig. 2. Cyclic voltammograms at scan rate 100 mV s^{-1}, BR buffer pH 9, E_{ad}=0 V, (1) blank sample (t_{ad}=60 s); (2) 1.0×10^{-6} mol L^{-1} AO solution (t_{ad}=0 s); (3) 1.0×10^{-6} mol L^{-1} AO solution (t_{ad}=60 s)

The results showed that, the peak current of AO having preconcentration step (curve 3) was higher than that of curve 2, which indicated that AO was adsorptive on the GCE surface.

On the other side, the voltammograms were recorded 5 cyclic repetitive at the same GCE surface. As show in figure 3, the repetitive anodic peak current (curve 2, 3, 4, 5) reduced rapidly compared to the first anodic cycle, which may be explained by desorption of the AO on the GCE surface.

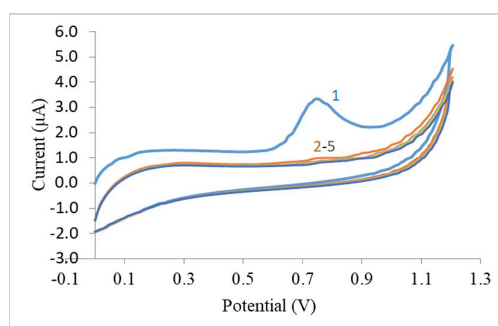


Fig. 3. Cyclic voltammograms at scan rate 100 mV s^{-1}, BR buffer pH 9, E_{ad}=0 V for 1.0×10^{-6} mol L^{-1} AO solution with t_{ad}=60 s (1); (2-5) repetitive cyclic of (1) at the same glassy carbon electrode

The cyclic voltammograms depending on pH was studied in various BR buffers from 8.0 to 11.0. The results show that anodic peak was not irreversible and peak potential shifted to a more negative value when pH increased which demonstrated participation of protons in the electrode reaction [11].

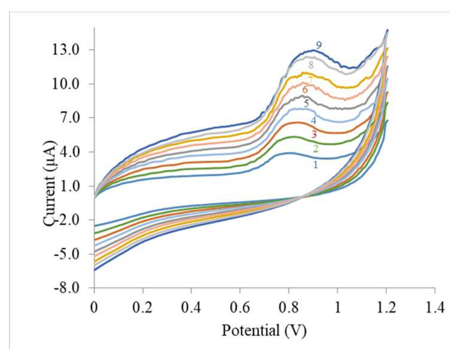


Fig. 4. Cyclic voltammograms for 1.0×10^{-6} mol L^{-1} auramine O in BR buffer pH 9 with increased scan rate: (1) 100; (2) 150; (3) 200; (4) 250; (5) 300; (6) 350; (7) 400; (8) 450; and (9) 500 mV s^{-1}; E_{ad}=0 V, t_{ad}=30 s

The effect of scan rate on the oxidation peak potential (E_{pa}) and current (I) of AO at GCE was examined by cyclic voltammetry by varying the sweep rate from 100 to 500 mV s^{-1}. Figure

4 indicates the cyclic voltammograms of 10^{-6} mol L^{-1} AO in BR buffer solution pH=9.0 at a scan rate ranging between 100 and 500 mV s^{-1}. The electrode reaction was irreversible as shown from the lack of a reduction peak in the cyclic voltammogram.

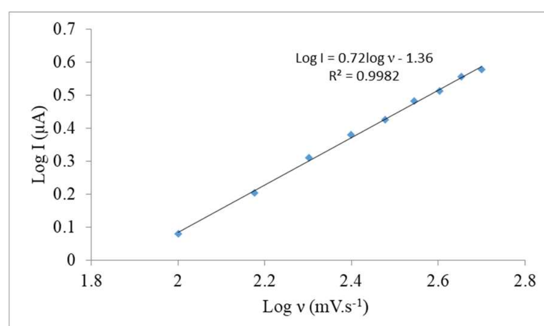
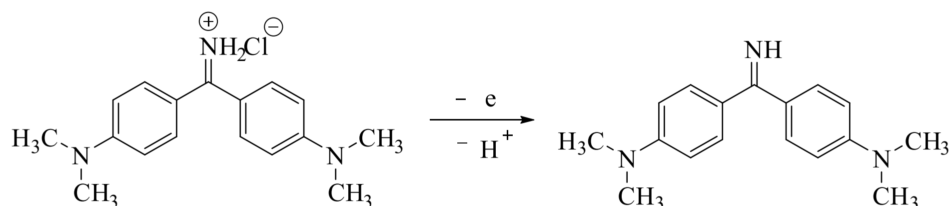


Fig. 5. Cyclic voltammetry current (log I_p) *vs.* logν plot for 1.0×10^{-6} mol L^{-1} AO in BR buffer pH 9 of different scan rate from 100 mV s^{-1} to 500 mV s^{-1}, E_{ad}=0 V, t_{ad}=30 s

The peak current (I_p) increased linearly with scan rates of 100-500 V s^{-1} according to the equation $\log I_p = 0.72 \log \nu - 1.36$; R^2=0.9982 (figure 5). For such a relation, if the slope value of equation was 1.0 then the electrochemical reaction occurred on the ideal surface of GCE and the slope value of 0.5 was reaction kind in the solution [12]. However, all most voltammetric properties of the surface limited species was not ideal [13]. From experimental results in figure 5, the slope value (0.72) of equation log I_p *vs.* log υ plot indicated that auramine O had an adsorptive process onto the GCE surface. Moreover, when the scan rate increased, the peak potential (E_p) shifted to more negative values which confirmed that the oxidation process of AO was irreversible [14]. The electrode process can be presented by the following reaction mechanism.



This electrode process of auramine O at glassy carbon electrode was consistent with the result of the section 3.2.1.

3.2. Differential pulse adsorptive stripping voltammetry

3.2.1. Effect of pH

pH is very important factors because of affecting the stability of the analyte, peak potential, peak current and its anodic oxidation. The results showed that, pH had affected to both peak current and potential, when pH was lower than 7 values then oxidation peak current was not observed. The oxidation peak appeared when pH was higher than 7. The highest oxidation peak current achieved at pH 10.0 so we chose as optimal pH value for subsequent research (figure 6). Moreover, the figure 7 showed that, the oxidation peak potential of AO shifted to lower values and linear dependence against pH of the solution. The regression equation was E_{pa}=-0.057 pH+1.239 (R^2=0.992). The slope value of dE_{pa}/dpH plot as 0.059 V pH^{-1} is very close to 0.0592/n V pH^{-1} with n=1, corresponding to the one H^+ per one electron stoichiometry.

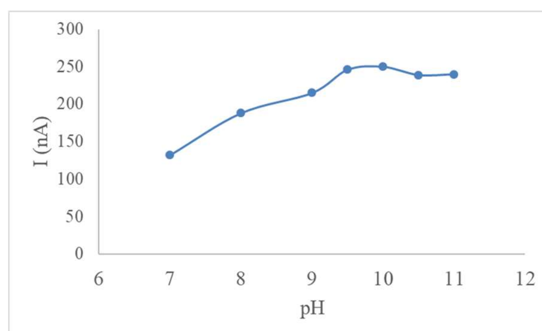


Fig. 6. Effect of pH (7.0-11.0) on the anodic current for 1.0×10^{-7} mol L^{-1} AO, E_{ad}=- 0.3 V, t_{ad}=60 s, scan rate 20 mV s^{-1}

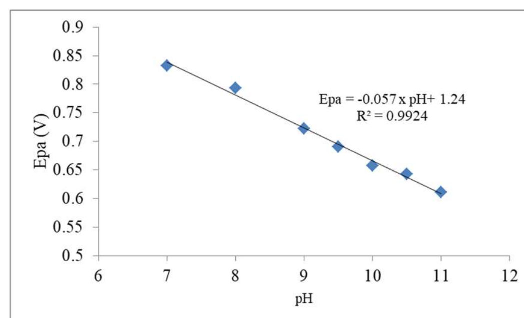


Fig. 7. Effect of pH (7.0 - 11.0) on the anodic peak potential (E_{pa}) of AO 1.0×10^{-7} mol L^{-1}, E_{ad}=- 0.3 V, t_{ad}=60 s, scan rate 20 mV s^{-1}

3.2.2. Effect of adsorption potential (E_{ad})

The adsorption potential affected current in the adsorptive stripping voltammetry method, so we studied on conditions: AO concentration 1.0×10^{-7} mol L^{-1}, BR buffer solution of pH 10,

adsorption time 60 s, scan rate 20 mV s^{-1}, adsorption potential changed from -1.0 V to 0.5 V. The figure 8 indicated that, peak current depended on adsorption potential. At the adsorption potential was 0 V, the peak current was highest. So, the adsorption potential of 0 V was chosen to study.

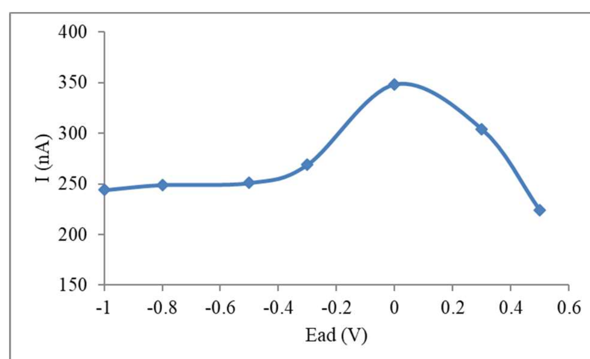


Fig. 8. Effect of adsorption potential (E_{ad}) on the anodic current for 1.0×10^{-7} mol L^{-1} AO, pH 10, t_{ad}=60 s, scan rate 20 mV s^{-1}

3.2.3. Effect of adsorption time (t_{acc})

The adsorption time depended on analyte concentration, so we investigated the effect of adsorption time on peak current with conditions: BR buffer of pH 10.0, concentration of 1.0×10^{-7} mol L^{-1} AO. As show in figure 9, the peak current of AO increased linearly with an increase in the adsorption time from 0 s to 90 s. If we increased time longer than 90 s, the peak current did not increase linearity, the GCE surface was reached saturation, indicating that AO has adsorbed single-coated on the GCE. Therefore, with n×10^{-7} mol L^{-1} AO solution, the adsorption time was chosen 90 s for determination of AO.

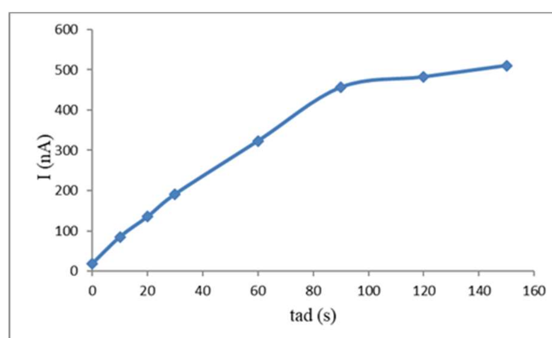


Fig. 9. Effect of adsorption time on the anodic current for 1.0×10^{-7} mol L^{-1} AO, pH=10.0, E_{ad}=0 V, scan rate 20 mV s^{-1}

3.2.4. Effect of other parameters

To achieve good results such as high sensitivity and selective, several instrumental parameters such as scan rate, pulse amplitude were investigated for 1.0×10^{-7} mol L^{-1} of AO solution in BR buffer at pH 10.0 in this study. When the scan rate and pulse amplitude increased, the peak current increased but peak width increased. So, we chose the scan rate of 25.0 mV s^{-1} and a pulse amplitude of 50 mV to study method to determine AO in food samples.

3.3. Validation of method

We evaluated a developed analytical method before applications to determine auramine O in chicken sample through the stability, linearity, sensitivity, repeatability, precision, recovery for the developed AdSV method.

3.3.1. Stability of standard solution

The AO standard stock solution (1.0×10^{-3} mol L^{-1}) was preserved in the dark at 4 ºC for 3 months. The stability of 1.0×10^{-5} mol L^{-1} of AO solution was evaluated by the proposed method. The results obtained to show that the peak potential and peak current of AO did not change at least 8 h.

3.3.2. Linearity of method

To evaluate the developed analytical procedure, we recorded the voltammograms with different concentration of AO. Peak currents increased linearly with the increasing amounts of AO from 1.0×10^{-8} mol L^{-1} to 20.0×10^{-8} mol L^{-1} following regression equation: I_p (nA)=41.06 $C_x\times10^{-8}$ (mol L^{-1}) +3.69, R^2=0,996 (n=5).

3.3.3. Sensitivity of method

The limit of detection (LOD) and limit of quantitation (LOQ) was used to evaluate the sensitivity. Base on calibration curves, we calculated the LOD, LOQ by formula 3×SD/b and 10×SD/b, where "SD" is the standard deviation of the intercept and "b" is the slope of the calibration curve [15]. Specifically, the LOD and LOQ of proposed method were 2.7×10^{-9} mol L^{-1} and 9.0×10^{-9} mol L^{-1}, respectively. The results indicated that the proposed method had highly sensitive, suitable for the analysis of AO in food samples.

3.3.4. Repeatability and precision of method

The repeatability of the voltammetric instrument was assessed through 10 consecutive measurements with the same standard solution 4.0×10^{-8} mol L^{-1} and 2.0×10^{-7} mol L^{-1} of AO

under the optimum conditions on the same day. The average peak current was 194 nA and 837 nA with RSD of 3.9% and 2.2%, respectively. These results confirmed that the proposed method had high repeatability and precision, we can apply to determine AO in food samples.

3.3.5. Recovery of method

The matrix components of the food samples are quite complicated. It may cause the wrong analysis result. To determine recovery of the method, we spiked the standard AO into chicken meat samples it was not AO at three concentrations of 1.82, 6.08 and 18.23 μg g^{-1}. The optimum treatment procedure was developed as mentioned in Section 2.4. Table 1 showed that, the recoveries of AO were found to be between 84.5-102.6% with RSD of 1.2-3.2% (n=5). The recovery was closeness to 100% so the developed method was very good, exactly and reliable.

Table 1. Recovery of AO spiked in chicken meat

Spiked Concentration into chicken sample (μg g^{-1})	Found Concentration in spiked samples (μg g^{-1})	Recovery (%)	RSD of Recovery (%)
1.82	1.72	102.6	3.2
6.08	5.56	91.5	1.2
18.23	15.9	84.5	1.7

3.5. Analytical application

We applied the proposed DPAdSV method to determine of AO content in spiked chicken meat and chicken meat samples which bought in markets. The chicken meat samples were treated as described in the experimental section 2.4 and analyzed under the optimum experimental conditions. The linear calibration graphs for determination of AO in spiked chicken meat samples were obtained in concentration from 1.81 μg g^{-1} to 24.29 μg g^{-1}. The variation of I_p versus AO concentration C was presented by the straight-line equation I_p (nA)=55.68×C (μg g^{-1})+2.98; r=0.996, n=6. The limits of detection (LOD) and quantitation (LOQ) of AO in spiked chicken meat were found to be 0.16 μg g^{-1} and 0.53 μg g^{-1}, respectively. From obtained results, we applied this procedure for determination of auramine O in chicken samples. We bought the chicken meat at Truong Dinh, Nguyen Cao, Khuong Dinh markets and pretreated samples as described in Section 2.4. We added AO concentration into some chicken meat samples to check recovery of method. The concentration of auramine O in chicken and spike chicken samples were analysed by DPAdSV and Ultra Performance Liquid Chromatography- tandem Mass Spectrometry method (UPLC-MS/MS). The results were presented in Table 2.

Table 2. The results of AO content in food product collected from local market

Samples	Spiked AO Concentration (μg g^{-1})	AdSV assay* (μg g^{-1}) found	UPLCMS/MS, (μg g^{-1}) found
Vandien 1	0	<LOD	<LOD
Vandien 1	6.08	5.75	5.87
Vandien 1	9.12	7.61	8.55
Vandien 2	0	<LOD	<LOD
Quynhdo 1	0	<LOD	<LOD
Quynhdo 2	0	<LOD	<LOD
Quynhdo 2	2.43	2.23	2.57
Quynhdo 2	3.04	3.51	2.69
Nguyencao	0	<LOD	<LOD
Truongdinh	0	<LOD	<LOD
Khuongdinh	0	<LOD	<LOD

*Results are the means of three replicate determinations

Comparison studies: In order to validate the experimental data in determining some chicken meat samples, we sent some chicken meat sample to the laboratory of Vinacert certification and inspection joint stock company. The auramine O content in samples was determined by UPLC-MS/MS method with UPLC conditions being Acquit UPLC BEH C18 column (i.d.: 2.1mmx50 mm; partial size 1.7 µm, Water) using a multistep gradient elution with mobile phase including 5 mM ammonium acetate (pH 3.0 with formic acid) and methanol [7].

The measured results showed that, both Adsorptive Stripping Voltammetry and Ultra Performance Liquid Chromatography- tandem Mass Spectrometry methods had not detected auramine O content in meat chicken samples. The average results obtained with the DPAdSV are the same with UPLC-MS/MS method. However, the DPAdSV method has a lot of advantages such as low running costs, no expensive, simple, fast.

4. CONCLUSION

The anodic differential pulse adsorptive stripping voltammetry method was investigated to determine auramine O in food sample. With experimental conditions, the electrochemical oxidation process of AO was an irreversible and adsorbed on the glassy carbon electrode surface. The developed DPAdSV method is sensitive, selective, repeat, precise and accurate, simple, easy to use for determination of AO with high recoveries from 84.5% to 102.6%. The

method can be applied to determine auramine O in chili sauce, marinated bamboo shoots, feed, fermented vegetable samples.

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