

Voltammetric Sensors for the Simultaneous Quantification of Natural Phenolics in Coffee and Citrus Juices [†]

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[†] Presented at the 4th International Electronic Conference on Applied Sciences, 27 October–10 November 2023; Available online: <https://asec2023.sciforum.net/>.

Abstract: Phenolic antioxidants of various groups are important nutrients in the human diet, providing positive health effects. Nevertheless, these effects are dose dependent and require the control of natural phenolic contents in their sources. Coffee and citrus juices containing significant amounts of hydroxycinnamic acids and flavanones, respectively, are among the most widely consumed beverages all over the world. The electroactivity of phenolics allows the application of voltammetric sensors for quantification purposes. Highly sensitive and selective voltammetric sensors for the simultaneous quantification of hydroxycinnamic (caffeic (CA), ferulic (FA), and *p*-coumaric(*p*-CA)) acids and flavanones (hesperidin and naringin) have been developed for the first time using glassy carbon electrodes modified with single-walled carbon nanotubes functionalized via polyaminobenzene sulfonic acid (f-SWCNTs) and polymeric coverages from triphenylmethane dyes (phenol red (PR) or aluminon). Polymeric layers have been obtained in potentiodynamic modes. The conditions of the dye's electropolymerization have been optimized using the voltammetric response of hydroxycinnamic acids or flavanone mixtures. Three separated oxidation peaks of CA, FA, and *p*-CA at the electrode with polyPR as well as hesperidin and naringin at the polyaluminon-modified electrode have been observed. The oxidation currents are significantly increased comparing those at the bare glassy carbon (GCE) and carbon nanotube-modified electrodes. Both sensors provide a highly sensitive response to target analytes in the differential pulse voltammetric mode. Other natural phenolics of various classes do not affect the response of the sensors developed to the target analyte. The quantification of hydroxycinnamic acids in coffee and flavanones in orange and grapefruit juices has been successfully realized.

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Citation: Zhupanova, A.; Ziyatdinova, G. Voltammetric Sensors for the Simultaneous Quantification of Natural Phenolics in Coffee and Citrus Juices. *Eng. Proc.* **2023**, *56*, 69. <https://doi.org/10.3390/ASEC2023-15314>

Academic Editor: Catarina Simões

Published: 26 October 2023



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Keywords: food analysis; natural phenolics; electrochemical sensors; electropolymerization; triphenylmethane dyes; simultaneous quantification

1. Introduction

Phenolic antioxidants of various groups are important nutrients in the human diet providing positive health effects [1]. Nevertheless, these effects are dose dependent and require the control of natural phenolic contents in their sources [2]. Coffee and citrus juices containing significant amounts of hydroxycinnamic acids [3] and flavanones [4], respectively, are among the most widely consumed beverages all over the world.

The electroactivity of phenolics allows the application of voltammetric sensors for quantification purposes. The possibility of the simultaneous determination of structurally related phenolic of the same class is one of the most important problems in modern electroanalysis that can be partially solved using sensors based on chemically modified electrodes [5]. Hydroxycinnamic acids and flavanones are less studied analytes from this point of view. There are several voltammetric sensors for the simultaneous determination of hydroxycinnamic acids (Table 1), while flavanones are out of consideration.

Table 1. Voltammetric sensors for the simultaneous quantification of hydroxycinnamic acids.

Sensor	Method	Hydroxycinnamic acid	Detection Limit (μM)	Linear Dynamic Range (μM)	Ref.
Carbon nanofiber-based screen-printed electrode	CV *	CA	0.239	10–1000	[6]
		FA	0.233	10–1000	
Poly(Sunset Yellow)/Multi-walled carbon nanotubes/GCE	DPV **	Chlorogenic acid	0.076	0.10–4.0	[7]
		FA	0.098	0.5–4.0	
WS ₂ flakes decorated with catechin-capped gold nanoparticles and carbon black/screen-printed electrode	DPV	CA	0.09	0.4–112.5	[8]
		Sinapic acid	0.36	0.7–125.0	
		<i>p</i> -CA	0.39	1.4–93.7	

* Cyclic voltammetry. ** Differential pulse voltammetry.

Among various types of electrode surface modifiers, electropolymerized coatings based on the compounds containing phenol fragments in the structure have been of interest as an effective sensing layer for natural phenolics [9].

Novel voltammetric sensors with high sensitivity and selectivity have been developed using f-SWCNTs and poly(triphenylmethane dyes) as a sensing layer for the simultaneous quantification of hydroxycinnamic acids (CA, FA, and *p*-CA) and flavanones (hesperidin and naringin) in coffee and citrus juices.

2. Materials and Methods

Hydroxycinnamic acids (98% CA, 98% *p*-CA from Sigma (Steinheim, Germany), 99% FA from Aldrich (Steinheim, Germany)) and flavanones (94% hesperidin and 95% naringin from Sigma-Aldrich (Germany)) were used as analytical standards. Stock solutions of analytes (10 mmol L⁻¹ (0.40 mmol L⁻¹ for hesperidin)) were prepared in ethanol (rectificate) for hydroxycinnamic acids and in methanol (c.p.) for flavanones. The exact dilution was used to obtain a less concentrated solution prior to the measurements.

Homogeneous 1.0 mg mL⁻¹ suspension of polyaminobenzene sulfonic acid functionalized single-walled carbon nanotubes (f-SWCNTs) ($\varnothing \times l$ was 1.1 nm \times 0.5–1.0 μm) from Sigma-Aldrich (Steinheim, Germany) was prepared in dimethylformamide using 30 min sonication in the ultrasonic bath (WiseClean WUC-A03H) (DAIHAN Scientific Co., Ltd., Wonju-si, Republic of Korea).

Standard 10 mM solutions of monomers were prepared in methanol for aluminon (Sigma-Aldrich, Steinheim, Germany) and in ethanol for the phenol red (PR) (Sigma-Aldrich (St. Louis, MO, USA)).

Potentiostat/galvanostat Autolab PGSTAT 302N with the FRA 32M module (Metrohm B.V., Utrecht, The Netherlands) and NOVA 1.10.1.9 software were used. All measurements were carried out in the 10 mL glassy electrochemical cell. The working bare GCE (\varnothing 3 mm from CH Instruments, Inc., Bee Cave, TX, USA) or a modified electrode and a counter platinum electrode were used. The potentials were measured against Ag/AgCl reference electrode.

“Expert-001” pH meter (Econix-Expert Ltd., Moscow, Russia) supplied with the glassy electrode was applied.

3. Results and Discussion

3.1. Effect of Electropolymerization Conditions on the Voltammetric Response of Target Analyte Mixtures at the Modified Electrode

The insulating nature of electropolymerized triphenylmethane dyes requires an increase in the electrode conductivity that is successfully provided by carbon nanomaterials as support for further polymeric coverage deposition. f-SWCNTs were drop cast at the GCE surface (2.0 μL), and the evaporation of the solvent to dryness at the ambient conditions was performed. Then, the electropolymerization of aluminon or PR was conducted in the potentiodynamic mode. Conditions of the dye’s electropolymerization have been opti-

mized using the voltammetric response of hydroxycinnamic acids or flavanone mixtures and summarized in Table 2.

Table 2. Triphenylmethane dye electropolymerization conditions provide the best response to natural phenolic mixture.

Electropolymerization Conditions	Monomer	
	Aluminon	PR
Supporting electrolyte	0.1 M NaOH	0.1 M NaOH
pH	13	13
Monomer concentration (μM)	100	75
Number of cycles	10	10
Electrochemical window (mV)	+100 to +800	−300 to +1200
Potential scan rate (mV s^{-1})	100	75

Clear separated oxidation peaks of CA, FA, and *p*-CA at the electrode with polyPR electrode in Britton–Robinson buffer (BRB) at pH 2.0, as well as hesperidin and naringin at the polyaluminon-modified electrode in phosphate buffer (PB) at pH 7.0, have been observed under the conditions of cyclic voltammetry. The oxidation currents are significantly increased, indicating the high sensitivity of the electrode towards hydroxycinnamic acids and flavanones (Table 3). Only the first oxidation peak parameters are presented for the hesperidin and naringin.

Table 3. Voltammetric characteristics of hydroxycinnamic acids and flavanones at the polymer-based electrodes ($n = 5$; $P = 0.95$).

Sensor	Analyte	E_{ox} (mV)	I_{ox} (μA)
GCE	Hesperidin	544	0.025 ± 0.001
	Naringin	745	0.0070 ± 0.0003
f-SWCNTs/GCE	Hesperidin	505	0.123 ± 0.005
	Naringin	705	0.104 ± 0.003
Polyaluminon/f-SWCNTs/GCE	Hesperidin	498	0.25 ± 0.01
	Naringin	705	0.19 ± 0.01
GCE	CA	530	0.150 ± 0.008
	FA	760	0.110 ± 0.006
	<i>p</i> -CA	940	0.080 ± 0.004
	CA	523	0.200 ± 0.007
f-SWCNTs/GCE	FA	753	0.180 ± 0.005
	<i>p</i> -CA	954	0.110 ± 0.003
	CA	524	0.61 ± 0.02
PolyPR/f-SWCNTs/GCE	FA	715	0.54 ± 0.01
	<i>p</i> -CA	906	0.53 ± 0.01

3.2. Quantification of Analytes in Differential Pulse Mode

Both sensors provided a highly sensitive response to target analytes in the differential pulse voltammetric mode (Figure 1). Taking into account the shape of voltammograms for the mixture of hydroxycinnamic acids, the peak area was used for calibration plot fitting. The analytical ranges of $0.10\text{--}2.5 \mu\text{mol L}^{-1}$ and $2.5\text{--}100 \mu\text{mol L}^{-1}$ for CA, $0.10\text{--}2.5 \mu\text{mol L}^{-1}$ and $2.5\text{--}50 \mu\text{mol L}^{-1}$ for both FA and *p*-CA s using polyPR-modified electrode were obtained. The detection limits of 48, 22, and 38 nmol L^{-1} were achieved for the CA, FA, and *p*-CA, respectively. Polyaluminon-based sensors allowed the determination of $0.10\text{--}2.5$ and $2.5\text{--}25 \mu\text{mol L}^{-1}$ of both flavanones. The detection limits were calculated as 29 and 20 nmol L^{-1} , respectively. The analytical characteristics obtained using polymer-based sensors are meaningful and improved compared to those presented in Table 1.

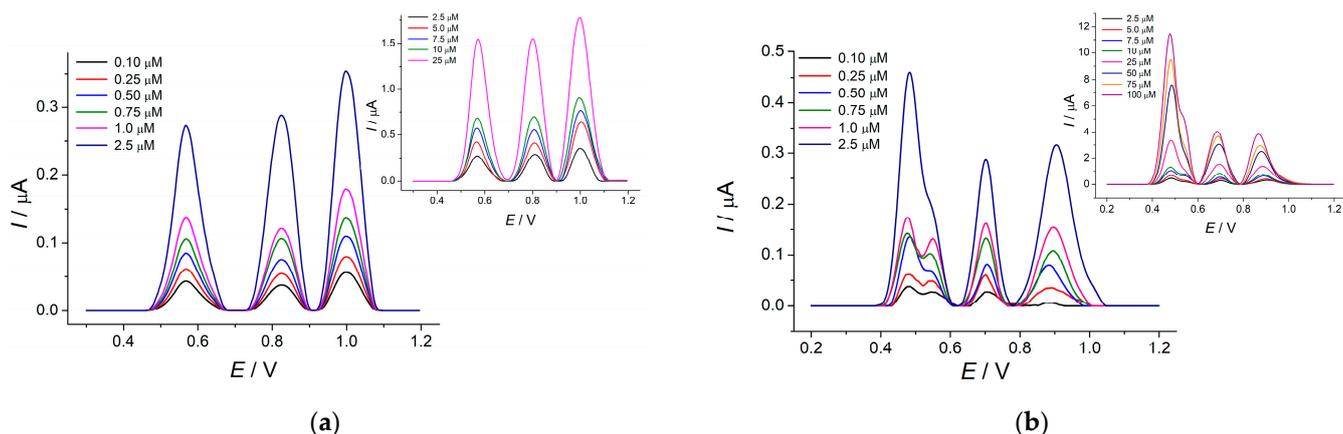


Figure 1. Baseline-corrected response of polymer-based sensors to the natural phenolics in differential pulse mode: (a) flavanons equimolar mixtures at the polyaluminum/f-SWCNT/GCE in PB at pH 5.0. $\Delta E_{\text{pulse}} = 0.100$ V, $t_{\text{pulse}} = 0.025$ s, and $\nu = 0.010$ V s⁻¹; (b) equimolar mixtures of CA, FA, and *p*-CA at the polyPR/f-SWCNT/GCE in BRB at pH 2.0. $\Delta E_{\text{pulse}} = 0.050$ V, $t_{\text{pulse}} = 0.025$ s, and $\nu = 0.010$ V s⁻¹.

Selectivity in the presence of other natural phenolics has been achieved. A 10-fold excess of gallic, caffeic, and chlorogenic acids, 5-fold excess of tannic acid, 1.0 μM catechin, quercetin, and rutin, <1.0 μM of FA, sinapic acid, and *p*-CA did not affect the determination of hesperidin and naringin at the polyaluminum-based sensor. The polyPR-based sensor is less selective toward hydroxycinnamic acids in the presence of other phenolics. Chlorogenic acid is oxidized at the same potential as CA; therefore, the total contents of these acids are determined in the case of real samples containing both compounds.

The quantification of hydroxycinnamic acids in espresso coffee and flavanones in orange and grapefruit juices has been successfully realized. Recovery values are close to 100%, indicating the absence of matrix effects and the practical applicability of the sensors developed. The data obtained are presented in Figure 2.

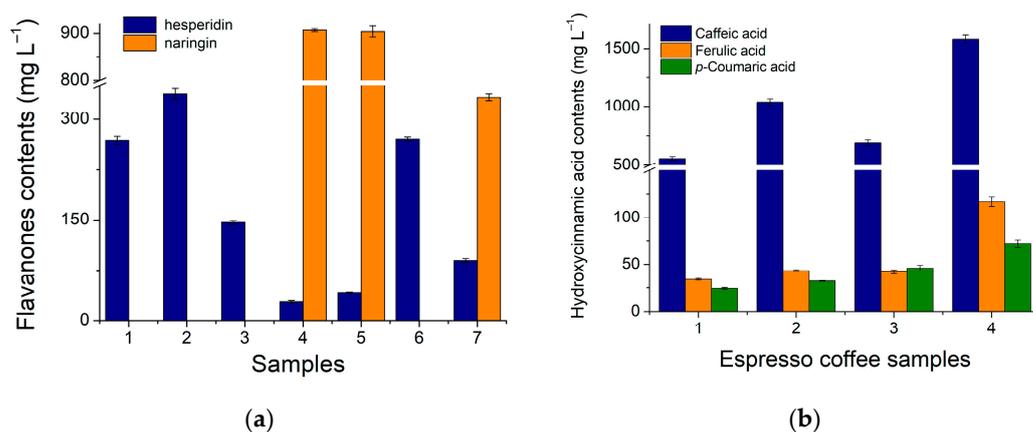


Figure 2. Determination of natural phenolics in beverages: (a) flavanones contents in orange (samples 1–3 (commercial) and 6 (fresh)) and grapefruit juices (samples 4, 5 (commercial) and 7 (fresh)) using polyaluminum/f-SWCNT/GCE; (b) hydroxycinnamic acid contents in espresso coffee using polyPR/f-SWCNT/GCE.

Author Contributions: Conceptualization, G.Z.; methodology, A.Z. and G.Z.; validation, A.Z.; investigation, A.Z.; writing—original draft preparation, A.Z. and G.Z.; writing—review and editing, G.Z.; visualization, A.Z. and G.Z.; supervision, G.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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