Food Chemistry 325 (2020) 126787

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Evaluation of antioxidant activity of chlorogenic acids and coffee extracts by an electrochemical DNA-based biosensor

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ARTICLE INFO

Chemical compounds in this article:
5-CQA (PubChem CID 5280633)
4-CQA (PubChem CID 9798666)
3-CQA (PubChem CID 1794427)
3,4-diCQA (PubChem CID 5281780)
3,5-diCQA (PubChem CID 6474310)
4,5-diCQA (PubChem CID 6474309)
CFA (PubChem CID 689043)

Keywords:
Electrochemical DNA-based biosensor
Oxidative DNA degradation
Antioxidant activity
Chlorogenic acids
Coffee extracts

ABSTRACT

Sensitivity of dsDNA structure towards OH˙ radicals as the pro-oxidants has been utilized as the detection principle of an analytical procedure applied for the first time to the evaluation of antioxidant activity (AOA) of 6 chlorogenic acids (CGAs) and extracts of 10 coffees. A nanostructured electrochemical DNA-based biosensor was prepared using a commercial electrode assembly and treated in the DNA cleavage agent formed by the Fenton type reaction. An addition of CGAs and aqueous coffee extracts significantly diminishes the degree of DNA degradation determined using cyclic voltammetry (CV) with the redox indicator $[Fe(CN)_6]^{3-/4-}$. The AOA decreases in order caffeic acid, CFA, > caffeoylquinic acids, CQAs, > dicaffeoylquinic acids, diCQAs, exhibiting the relative portion of survived DNA of about 71%, 70% and 69%, respectively, and of about 72% for *C. robusta*, Cherry, India (green bean) to 49% for Nescafé Espresso. Mechanisms of antioxidative properties are discussed.

1. Introduction

Chlorogenic acid (CGA) is a trivial name used for major phenolic components found in coffee. The CGA content represents 5–10% of coffee beans, which is a much larger amount than caffeine (1–2%). In fact, CGAs present in coffee are mainly formal esters of quinic acid with caffeic, ferulic, or coumaric acids. Significant amounts of CGA isomers (in positions 3, 4, and 5 of quinic acid) are usually found in green and processed coffees, namely caffeoylquinic acids (CQAs), di-caffeoylquinic acids (di-CQAs), ferulyolquinic acids (FQAs), *p*-coumaroylquinic acids (*p*-CoQAs), and others (Farah & Lima, 2019; Kuhnert, Karaköse, & Jaiswal, 2012). An intake of CGAs through coffee drinking has many beneficial effects on human health, like antioxidative, antidiabetic, anticarcinogenic, cardioprotective, antibacterial effects, etc.. Antioxidants play an important role as a health-protecting factor, limiting the biomolecules lesions caused by reactive oxygen species (ROS).

For the investigation of antioxidant activity (AOA) of complex food matrices such as coffee, various methods have been developed including colorimetric tests using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt assay (ABTS), fluorescence recovery after photobleaching assay (FRAP), and the Cupric Reducing Antioxidant Capacity method (CUPRAC) (Apak et al., 2013; Górnaś et al., 2016; Jeszka-Skowron, Stanisz, & De Peña, 2016; Ngamsuk, Huang, & Hsu, 2019). However, AOA of the CGAs isomers was only rarely investigated: that of 5-CQA in only one paper by the ABTS and FRAP methods (Szewczyk, Morawiak, Narczyk, Pakulski, & Urbanczyk-Lipkowska, 2018), and that of several CGAs isomers by the DPPH and ABTS methods (Xu, Hu, & Liu, 2012). The spectrophotometric methods have some disadvantages because their results are strongly dependent on very different experimental conditions. Therefore, these methods often give very different results for the same sample. Moreover, they are not based on reactions between the antioxidants and ROS and can not get direct information on the protective effect of antioxidants against an attack of ROS on biosubstrates such as DNA and others.

Electrochemical methods (Newair, Kilmartin, & Garcia, 2018; Ziyatdinova, Aytuganova, Nizamova, & Budnikov, 2013) and biosensors based on sensitivity towards ROS have been described to evaluate the antioxidant capacity in the food industry (Akyuz, Baskan, Tutem, & Apak, 2020; Calas-Blanchard, Cortina-Puig, Barthelmebs, &

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https://doi.org/10.1016/j.foodchem.2020.126787
Received 29 November 2019; Received in revised form 20 March 2020; Accepted 9 April 2020
Available online 24 April 2020

Noguer, 2012). Various electrochemical DNA-based biosensors have been successfully applied to the detection of DNA damage induced by ROS (Fojta, Danhel, Havran, and Vyskocil, 2016; Hajkova, Barek, & Vyskočil, 2017), as well as to the determination of the AOA of various antioxidants in the form of pure chemical compounds. The DNA modified screen-printed carbon electrode (DNA/SPCE) and carbon paste electrode (DNA/CPE) were used for the investigation of antioxidative properties of quercetin, rutin, catechin and epigallocatechin gallate (Labuda, Bučková, Heilerová, Šilhár, & Štepánek, 2003), gallic acid, caffeic acid, and trolox (Skeva & Girousi, 2012), rutin, caffeic acid, ferulic acid, gallic acid, chlorogenic acid and sinapic acid (Šimková, Beinrohr, & Labuda, 2009).

Electrochemical DNA-based biosensors were also very successfully used to evaluate the AOA of some food samples, mainly beverages. The biosensor constructed by an immobilization of purine bases (guanine and adenine) on a glassy carbon electrode (GCE) was used for the AOA determination of flavoured waters (Barroso, Delerue-Matos, and Oliveira, 2012). The DNA-based biosensors were utilized to investigate antioxidative properties of various tea extracts (Ferancová et al., 2004; Šimková et al., 2009; Ziyatdinova, Galandova, & Labuda, 2008), fruit juices (Ziyatdinova & Labuda, 2012), beer, coffee, and black tea extracts (Hlavatá, Vyskočil, Beníková, Borbélyova, & Labuda, 2014), as well as white wines (Svitková, Steffelová, Blaškovičová, & Labuda, 2015). To improve the sensitivity of carbon-based electrode substrates, particularly cheap ones like various carbon inks and pastes, an electrically conductive interface between the bare electrode and the DNA has been utilized. For this purpose, new sensor generation with multi-walled carbon nanotubes (MWCNT) becomes quite popular in electroanalytical chemistry (Ahammad, Lee, & Rahman, 2009; Hlavatá, Benikova, Vyskocil, & Labuda, 2012; Oliveira & Morais, 2018). Depending on their carbon nanotubes structure, single walled carbon nanotubes (SWCNT) were reported to exhibit also advantageous properties even over those of MWCNT (Ahammad et al., 2009). Both, MWCNT and SWCNT covalently functionalized by the carboxylic groups are known to exhibit higher hydrophilicity and water miscibility which is advantageous for the work with them and desired applications (Ahammad et al., 2009).

As it follows from this overview, the AOA evaluation of extracts of food samples is of great importance. Nevertheless, for the coffee samples and the individual CGAs isomers this parameter was only rarely investigated by the electrochemical DNA-based biosensors with an exception of those based on the bare SPCE (Šimková et al., 2009) and non-commercial MWCNT modified SPCE (Hlavatá et al., 2014; Ziyatdinova et al., 2008). This paper deals with a biosensor based on non-expensive commercial assambly containing the carboxyl functionalized single-walled carbon nanotubes (SWCNT-COOH) modified screen-printed carbon electrode (SPCE) where, according to the producer, the nanostructured interface improves an electrical conductivity of the carbon ink and retains the electrocatalytic properties of carbon nanotubes, thus enhances the sensitivity of the voltammetric measurement. The aims of this study were: (i) to prepare and characterize a simple, sensitive, and effective electrochemical DNA-based biosensor using the SWCNT-COOH/SPCE assembly for applications in the aqueous food samples like the coffee extracts, (ii) to propose an analytical procedure for the evaluation of AOA the CGAs isomers and aqueous coffee extracts prepared from commercial coffee samples, (iii) to characterise an influence of the chemical structure of the CGAs isomers on their antioxidant activity as well as to show the influence of the CGAs redox potential values and their content in the coffee samples.

2. Materials and methods

2.1. Chemicals and solutions

Salmon sperm double-stranded DNA (dsDNA) was purchased from Sigma-Aldrich, Germany. Stock solution of dsDNA (1 mg mL^{-1}) was prepared in a 0.1 mol L^{-1} phosphate buffer solution (PB) of pH 7.0 containing 0.01 mol L^{-1} NaCl, and stored at −4 °C. PB was prepared from Na_2HPO_4, NaH_2PO_4, and NaCl in Milli-Q water. $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were obtained from Merck, Germany. These chemicals were used for the preparation of redox indicator $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ solution, $c = 1{\cdot}10^{-3}$ mol L^{-1} in PB of pH 7.0. This redox system was used to detect the presence of immobilized DNA on the electrode, as well as the changes in DNA signals observed during the incubation of biosensor in the cleavage agent and in a mixture of cleavage agent and some of investigated CGAs isomers or coffee extracts. The Fenton reaction carried out in the mixture of $2.5{\cdot}10^{-4}$ mol L^{-1} H_2O_2 and $1{\cdot}10^{-6}$ mol L^{-1} of $CuSO_4$ was used for the $OH^{\cdot}$ radicals generation *in-situ*.

Caffeoylquinic acids 3-CQA, 4-CQA, and 5-CQA, were obtained from Sigma-Aldrich, USA, while dicaffeoylquinic acids 3,5-diCQA, 3,4-diCQA, and 4,5-diCQA were purchased from Chengdu Biopurity Phytochemicals Ltd., China. Caffeic acid (CFA) was purchased from Merck, Germany. Stock solutions of all CGAs and CFA were prepared in methanol and stored in a refrigerator at 4 °C. The chemical structures of investigated CGAs, their structural units (CFA and quinic acid), and the detailed structure of 5-CQA have been presented in Scheme 1 (Appendix A. Supplementary data).

2.2. Coffee samples

Ten different coffee samples were tested (see Table 2). Green and roasted beans *Coffea robusta*, Cherry, India, green and roasted beans *Coffea arabica*, Rio Minas, Brazil were acquired from Anamaria Company Croatia. Ground coffee Flatscher Olympia, 100% Arabica were acquired from Flatscher Company, Croatia. Ground coffee Guatemala, 100% Arabica, Franck Company, Croatia; instant coffee Nescafé Classic and Nescafé Espresso, Nestlé S.A., Switzerland; Jacobs Monarch and Jacobs Intense, Jacobs Douwe Egberts, Georgia, were purchased from the local market. The aqueous coffee extracts were prepared as described in our earlier studies (Šeruga & Tomac, 2014; Tomac & Šeruga, 2016; Tomac, Jakobek, & Šeruga, 2018; Tomac, Šeruga, & Beinrohr, 2017). Briefly, 1 g of coffee sample was overflowed by 100 mL of hot ultrapure water (80 °C). After mixing for 3 min with a magnetic stirrer, it was cooled to ambient temperature and filtered through filter paper prior analysis.

For the electrochemical measurements performed by DNA-biosensor, the green beans coffee extracts were diluted in 1:100 (v/v), while all other coffee extracts were diluted in 1:50 (v/v) with the supporting electrolyte (0.1 mol L^{-1} PB of pH 7.0 with 0.01 mol L^{-1} NaCl).

2.3. Equipments

The commercially available screen-printed carbon electrodes (SPCEs) modified with the carboxyl functionalized single-walled carbon nanotubes (SWCNT-COOH) were purchased from DropSens, Spain. The SWCNT-COOH/SPCEs are specially designed for the development of biosensor with an enhanced electrochemical active area and enhanced electron transfer properties. The SWCNT-COOH/SPCE assembly represents a three-electrode electrochemical arrangement consisting of SWCNT-COOH layer on carbon substrate as the working electrode, the carbon counter electrode, and a silver layer reference electrode. Electric contacts of all three electrodes were made of the silver. SWCNT-COOH/SPCE electrode assembly was connected to the potentiostat through a cable connector. The cyclic voltammetric measurements were performed by the Autolab PGSTAT-100 potentiostat using GPES version 4.9.005 software (Eco Chemie B.V., The Netherlands).

2.4. Preparation of the DNA biosensor

Prior to DNA biosensor preparation, SWCNT-COOH/SPCE electrode

was immersed into the redox indicator solution $[Fe(CN)_6]^{3-/4-}$, $c = 1{\cdot}10^{-3}$ mol L^{-1}, and the CV scan was performed within the potential range from −0.4 V to + 0.7 V and back to −0.4 V, by scan rate of 50 mV s^{-1} to get the CV response of the electrode without DNA layer. Then, the electrode was washed with Milli-Q water and leave for a few minutes to dry. The DNA biosensor was prepared according to optimized conditions by the application of 5 μL of dsDNA stock solution (c = 1 mg mL^{-1}) onto the surface of the working electrode (SWCNT-COOH/SPCE) and drying at room temperature for 30 min to complete the immobilization of DNA onto the electrode surface. Then, one CV scan was carried out at DNA/SWCNT-COOH/SPCE electrode from 0 V to + 1.4 V and back to 0 V by scan rate 100 mV s^{-1} in PB solution of pH 7.0. A worse developed CV picture of the negatively charge redox indicator at a negatively charges DNA surface has allowed to check that dsDNA is successfully immobilized (adsorbed) onto the working electrode surface.

2.5. Working procedure for the DNA damage detection and antioxidant activity evaluation

Prior to the DNA damage experiments, the CV scan with DNA/SWCNT-COOH/SPCE was performed in the redox indicator solution $[Fe(CN)_6]^{3-/4-}$, $c = 1{\cdot}10^{-3}$ mol L^{-1}, in 0.1 mol L^{-1} PB of pH 7.0 at the potential range from −0.4 V to + 0.7 V and back to −0.4 V by scan rate of 50 mV s^{-1}. At the detection of DNA damage, the same biosensor was rinsed with Milli-Q water and incubated in the cleavage agent (a solution containing the optimized composition of $2.5{\cdot}10^{-4}$ mol L^{-1} H_2O_2 and $1{\cdot}10^{-6}$ mol L^{-1} of $CuSO_4$) for a given time. After washing with Milli-Q water again, the CV scan in a redox indicator $[Fe(CN)_6]^{3-/4-}$ solution was performed using the experimental conditions as mentioned above. Fresh cleavage mixture was used for each experiment. To obtain the mean response values, the DNA damage experiments and the CV scans were repeated three times.

At the determination of the antioxidant activity, the same procedure was used, however, with the biosensor incubation in the cleavage agent with an addition of CGAs or aqueous coffee extracts. To compensate some differences in the biosensor responce due to the use of newly prepared sensor for each experiment, a relative portion of survived DNA (surv DNA, %) was expressed as the normalized biosensor responses, $\Delta I_{a,rel}$, $\Delta I_{c,rel}$ or $\Delta(\Delta E_{p,rel})$ calculated according to Eqs. (1)–(3). (Hlavatá et al., 2014; Ziyatdinova & Labuda, 2012):

$$\Delta I_{a,rel}/\% = (I_{a,surv\ DNA} - -I_{a,SWCNT\text{-}COOH/SPCE})/(I_{a,DNA} - -I_{a,SWCNT\text{-}COOH/SPCE}){\cdot}100 \quad (1)$$

$$\Delta I_{c,rel}/\% = (I_{c,surv\ DNA} - -I_{c,SWCNT\text{-}COOH/SPCE})/(I_{c,DNA} - -I_{c,SWCNT\text{-}COOH/SPCE}){\cdot}100 \quad (2)$$

$$\Delta(\Delta E_{p,rel})\ /\ \% = (\Delta E_{p,surv\ DNA} - -\ \Delta E_{p,SWCNT\text{-}COOH/SPCE}) /(\Delta E_{p,DNA} - -\ \Delta E_{p,SWCNT\text{-}COOH/SPCE}){\cdot}100 \quad (3)$$

where I_a and I_c are the CV anodic and cathodic current responses of $1{\cdot}10^{-3}$ mol L^{-1} redox indicator measured at its peak potential observed at the SWCNT-COOH/SPCE without DNA, and ΔE_p is the CV anodic to cathodic peak potential separation. The indexes DNA and surv DNA characterize the DNA biosensor before and after the incubation in cleavage agent (or mixture of cleavage agent and some of antioxidant), respectively. These normalized values were used to express the antioxidant activity of the CGAs solutions and coffee extracts under investigation against oxidative damage of the DNA induced by $OH^{\cdot}$ radicals.

2.6. Data treatment

Cyclic voltammograms (CVs) were analyzed using electrochemical software GPES version 4.9.005 (Eco Chemie B.V., The Netherlands) to get the electrochemical parameters (I_a, I_c, and ΔE_p). Figures of CVs have been done by OriginPro 8 software (OriginLab Corporation, USA). Drawing of histograms was done using Excel for Office 365 software (Microsoft Corporation, USA). Chemical structures have been drawn using ChemDraw 18.0 software (PerkinElmer Informatics, USA).

3. Results and discussion

3.1. Electrochemical behaviour of DNA/SWCNT-COOH/SPCE biosensor

As stated above, the DNA biosensor was prepared by the application of a dsDNA stock solution onto the commercial SWCNT-COOH modified working electrode. All steps of the biosensor preparation (namely the concentration and applied volume of the dsDNA stock solution as well as procedure of the DNA immobilization) and the biosensor application at the CV records (namely the scan rate) were optimized where the optimization criteria were obtaining the stable and reproducible CV curves of the $[Fe(CN)_6]^{3-/4-}$ indicator and a large shape difference of CVs for the bare working electrode, SWCNT-COOH/SPCE, and the DNA biosensor, DNA/SWCNT-COOH/SPCE. The optimized parameters have been utilized for further work.

Observing the DNA adsorption onto an electrode surface by any electrochemical method is the first necessary step in developing of the DNA-electrochemical biosensors (Diculescu, Chiorcea-Paquim, & Oliveira-Brett, 2005). In our case, one cyclic voltammetric (CV) scan at DNA/SWCNT-COOH/SPCE was carried out within the potential range from 0 V to + 1.4 V and back to 0 V with the scan rate of 0.1 V s^{-1} in PB solution of pH 7.0. The cyclic voltammogram (Fig. 1) shows two well known irreversible anodic peaks due to the oxidation of two purine nucleobases residues. The first peak (A1) at a potential of +0.83 V corresponds to the guanine residue, while the second one (A2) at +1.12 V to the adenine residue present in immobilized DNA molecule (Diculescu et al., 2005). The oxidation of the other two pyrimidine bases residues, thymine and cytosine, was not observed here due to their high oxidation potentials.

In difference to irreversible intrinsic signals of nucleobases, the reversible redox system $[Fe(CN)_6]^{3-/4-}$ can be utilized for a repeatable electrochemical indication of the amount of non-degradated DNA at DNA/SWCNT-COOH/SPCE (Labuda et al., 2010). The CV curve of the $[Fe(CN)_6]^{3-/4-}$ redox couple at the bare SWCNT-COOH/SPCE electrode (Fig. 2, curve e) shows a very well expressed anodic peak and cathodic counter-peak with the peak to peak potential separation ΔE_p of 0.107 V and the $I_{p,c}/I_{p,a}$ peak current ratio of 1.01 that correspond to a reversible redox behavior of the indicator at the electrode substrate used. On the other hand, the indicator CV response at DNA/SWCNT-

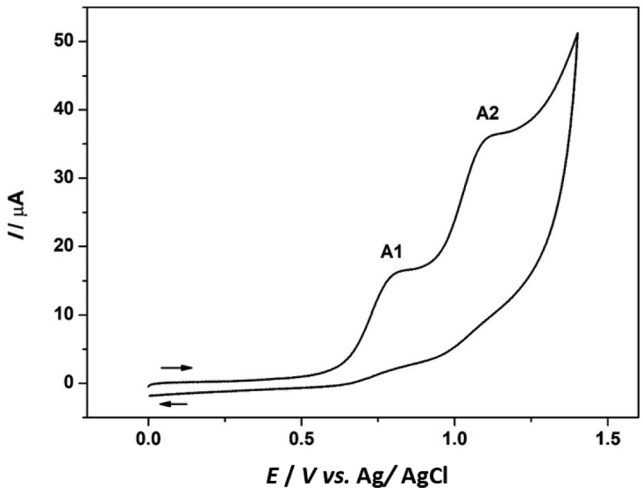

Fig. 1. Cyclic voltammogram of dsDNA immobilized on SWCNT-COOH/SPCE electrode recorded in 0.1 mol L^{-1} PB of pH 7.0 with 0.01 mol L^{-1} NaCl. Scan rate of 100 mV s^{-1}.

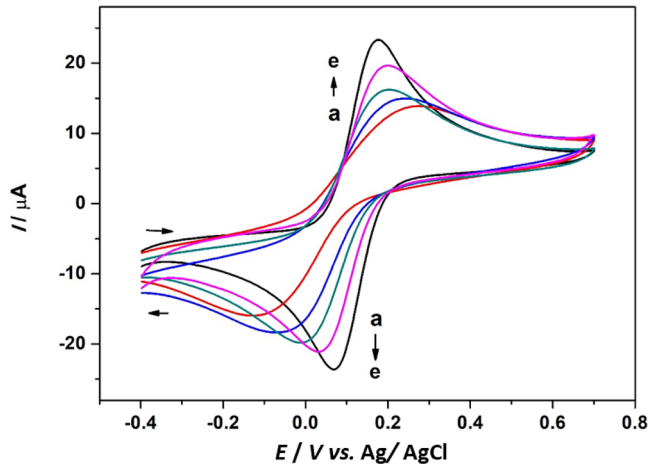

Fig. 2. Cyclic voltammograms of 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ in 0.1 mol L^{-1} PB solution of pH 7.0 with 0.01 mol L^{-1} NaCl, recorded at DNA/SWCNT-COOH/SPCE before (curve a) and after the incubation of the biosensor in the cleavage agent producing hydroxyl radicals for 2 min (curve b), 5 min (curve c), and 15 min (curve d). Curve (e) corresponds to the voltammogram recorded at a bare SWCNT-COOH/SPCE. Scan rate of 50 mV s^{-1}.

COOH/SPCE (curve a) indicates a significant decrease in currents and the increase in ΔE_p (0.405 V) due to an electrostatic repulsion effect between the negatively charged DNA backbone and the $[Fe(CN)_6]^{3-/4-}$ anions (Hlavatá et al., 2014; Šimková et al., 2009; Svitková et al., 2015).

3.2. Detection of the DNA oxidative damage induced by hydroxyl ($OH^{\cdot}$) radicals

For deep DNA damage, the $OH^{\cdot}$ radicals were produced by the Fenton reaction in the cleavage mixture of H_2O_2 and Cu(II) ions. The concentration of the reagents and the biosensor incubation time utilized were optimized in order to obtain a detectable effect on the DNA structure within a real time of experiment. Fig. 2 shows changes in the CV response of $[Fe(CN)_6]^{3-/4-}$ at DNA/SWCNT-COOH/SPCE after its incubation in cleavage agent for various immersion time: 2 min (curve b), 5 min (curve c), and 15 min (curve d). An increase of the $[Fe(CN)_6]^{3-/4-}$ peak currents and a decrease of the peak potential separation, ΔE_p, (from 0.306 V for 2 min to 0.171 V for 15 min of incubation) indicate a gradual degradation of the DNA layer as a result of the attack of $OH^{\cdot}$ radicals. The CV curves become to be better developed and similar to that observed at the electrode without DNA (curve e). Similar behaviour was reported by Ziyatdinova and Labuda (2012), Hlavatá et al. (2014), and Svitková et al. (2015). From the CVs, the normalized values of the biosensor responses $\Delta I_{a,rel}$, $\Delta I_{c,rel}$, and $\Delta(\Delta E_{p,rel})$ for an individual incubation time of 2, 5, and 15 min were calculated according to Eqs. (1)–(3). The relative portion of survived DNA (%) significantly decreases with incubation time, for instance, that based on $\Delta I_{a,rel}$ from 83% after 2 min, and 73% after 5 min, to only 38% after 15 min of incubation in the cleavage agent.

A number of reports deal with the deep damage of DNA induced by various ROS including hydroxyl radicals. The hydroxyl radicals induce different oxidative damage to DNA by different mechanisms. Thus, $OH^{\cdot}$ radicals react with the nucleobases of DNA by the addition reactions on the double −C═C− bonds in the structure of heterocyclic nucleobases, what generates different OH-adduct radicals. Further oxidation and reduction reactions of these adduct radicals finally lead to the opening of the heterocyclic nucleobase ring causing the damage to DNA structure (Dizdaroglu & Jaruga, 2012). On the other hand, $OH^{\cdot}$ radicals react with the sugar moiety of DNA by abstracting an H-atom from each of C-atoms. These processes result in the formation of different carbon-centred sugar radicals. Further reactions of these sugar radicals generate various sugar products, DNA strand breakage (breaks of sugar-phosphate bonds), and release of nucleobases from DNA, i.e. deep degradation of DNA (Dizdaroglu & Jaruga, 2012). Although many authors were investigated the mechanisms of DNA degradation processes induced by $OH^{\cdot}$ radicals (and other ROS), until now the nature of these processes is still not completely clear.

3.3. Evaluation of the antioxidant activity of chlorogenic acids

The DNA/SWCNT-COOH/SPCE biosensor was used for determination of the antioxidant activity of chlorogenic acids (CGAs) via the detection of the protective effect of CGAs towards the DNA oxidative damage induced by hydroxyl radicals. The experimental procedure and protocol were the same as in the case of tests of damage to DNA by cleavage agent only. The influence of the addition of 5-CQA as one of the CGAs isomers ($c = 1{\cdot}10^{-6}$ mol L^{-1}) into the cleavage agent solution on the CV responses of the redox indicator is shown on Fig. 3.

An increase of the $[Fe(CN)_6]^{3-/4-}$ redox indicator peak currents, and decrease of the peak potential separation, ΔE_p, (from 0.258 V for 2 min to 0.219 V for 15 min of incubation) can be seen. However, comparing to the CVs observed after incubation in the cleavage agent only (Fig. 2), the incubation in the presence of 5-CQA (Fig. 3) leads to lower values of the anodic and cathodic currents and higher values of ΔE_p, than those observed after incubation in the cleavage agent only, which clearly suggests that the DNA layer degradation is significantly decreased in the presence of 5-CQA acid as a strong antioxidant. In Fig. 4, the values of the normalized biosensor response, $\Delta I_{a,rel}$, obtained after the biosensor incubation in the cleavage agent only (column a) and in a mixture of cleavage agent and 5-CQA (column b) are compared. A significantly higher portion of survived DNA was found after the incubation in the mixture of cleavage agent and 5-CQA particularly at the long incubation time.

Table 1 summarized the values of relative portion of survived DNA after the DNA biosensor incubation in a mixture of the cleavage agent and some of the CGAs isomers for 15 min. The relative portion of survived DNA (surv DNA/%) expressed by individual normalized anodic and cathodic current responses are in good agreement. Something lower values indicated by the $\Delta(\Delta E_{p,rel})$ data were reported also by Šimková et al. (2009) for the AOA of some hydroxycinnamic acids. It can be concluded that all CGAs under investigation show significant antioxidant effect against the oxidative damage to DNA

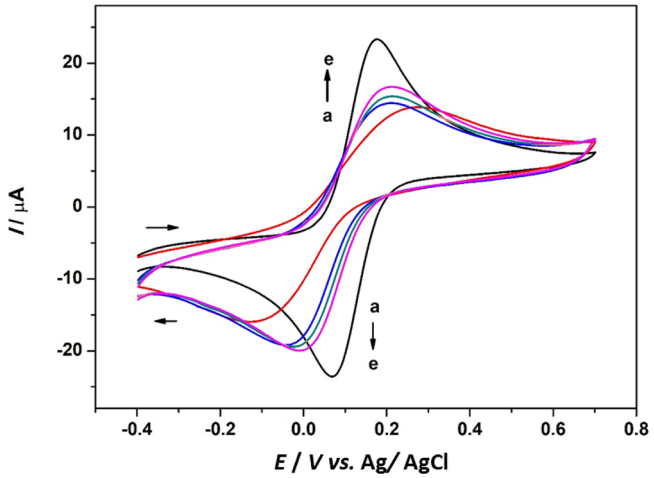

Fig. 3. Cyclic voltammograms of 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ in 0.1 mol L^{-1} PB solution of pH 7.0 with 0.01 mol L^{-1} NaCl, recorded at DNA/SWCNT-COOH/SPCE before (curve a) and after the incubation of the biosensor in the mixture of cleavage agent and 5-CQA, $c = 1{\cdot}10^{-6}$ mol L^{-1}, for 2 min (curve b), 5 min (curve c), and 15 min (curve d). Curve (e) corresponds to the voltammogram recorded at SWCNT-COOH/SPCE. Scan rate of 50 mV s^{-1}.

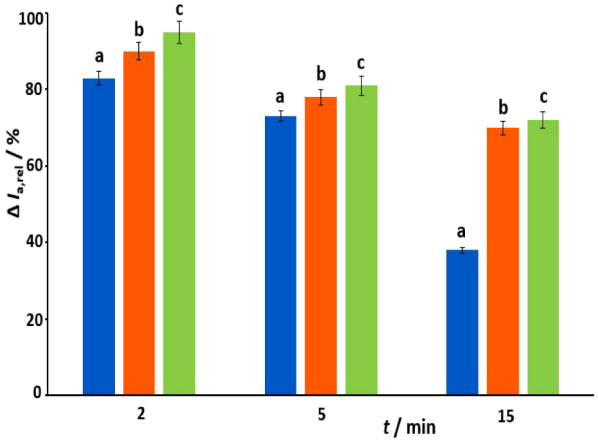

Fig. 4. Antioxidant activity of 5-CQA and *C. robusta* green beans extract evaluated by the normalized biosensor response values ($\Delta I_{a,rel}$) after the incubation of the biosensor in the cleavage agent only (column a), in the cleavage agent solution containing $1{\cdot}10^{-6}$ mol L^{-1} of 5-CGA (column b), and in the 1:100 (v/v) mixture of the cleavage agent and *C. robusta* green beans extract (column c) for a given incubation time.

Table 1
Antioxidant activity of different CGAs isomers towards the oxidative DNA damage evaluated by the normalized CV responses $\Delta I_{a,rel}$, $\Delta I_{c,rel}$, and $\Delta(\Delta E_{p,rel})$ to express the relative portion of survived DNA at the DNA/SWCNT-COOH/SPCE biosensor. For comparison, the voltammetric oxidation potential values are given. Conditions: Incubation of DNA/SWCNT-COOH/SPCE in cleavage agent or a mixture of cleavage agent and some of the CGAs isomers ($c = 1{\cdot}10^{-6}$ mol L^{-1}) for 15 min., the CV record in 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ in 0.1 mol L^{-1} PB of pH 7.0 with 0.01 mol L^{-1} NaCl.

	Relative portion of survived DNA/%			Oxidation potential
Antioxidant	$\Delta I_{a,rel}$	$\Delta I_{c,rel}$ AgCl[a]	$\Delta(\Delta E_p)_{rel}$	E_p/V vs. Ag/
No antioxidant	38.0	37.9	33.8	–
CFA	71.2	71.0	67.4	0.191
5-CQA	70.0	69.8	66.7	0.223
4-CQA	70.1	69.7	66.4	0.223
3-CQA	70.2	69.9	66.8	0.223
3,4-diCQA	68.8	68.3	65.3	0.229
3,5-diCQA	68.7	68.2	65.4	0.229
4,5-diCQA	68.5	68.1	65.2	0.229

-Results represent the mean value of three independent measurements ($n = 3$).
[a] Results obtained from Šeruga and Tomac (2014).

It seems that the position of esterification of caffeic acid on the quinic moiety (see Scheme 1) does not influence the AOA of CQAs and diCQAs as there is practically no difference in the its values found for the 5-CQA, 4-CQA, and 3-CQA isomers. On the other hand, the CQAs isomers (e.g. 5-CQA) have slightly lower AOA than their main constitutional moiety, the caffeic acid (CFA), and the diCQAs isomers show even lower AOA than the CQAs isomers. The AOA of CFA, CQAs and diCQAs (and also other polyphenols) strongly depends on the presence of catechol moiety/moieties in their structure. The presence of strong electron-withdrawing ester (-COOR) group(s) in the side chain of CQAs and diCQAs (Scheme 1) shifts the oxidation potentials of CQAs and diCQAs to more positive values in comparison to that of CFA and at diCQAs with two ester groups, this electron-withdrawing effect is more pronounced than at CQAs (Šeruga & Tomac, 2014; Tomac & Šeruga, 2016; Tomac et al., 2017). The higher oxidation potentials correspond to the more difficult electron transfer and to the lower antioxidant activity (Yang, Kotani, Arai, & Kusu, 2001). Thus, the observed AOA decrease in order CFA > CQAs > diCQAs (Table 1) can be explained by a similarity of processes of electrochemical oxidation of polyphenolic antioxidants (e.g. CQAs and diCQAs) and their reaction(s) with the ROS radicals.

There are two approaches to explain how the CGAs isomers as strong antioxidants significantly reduce the oxidative damage to DNA induced by the $OH^{\cdot}$ radicals. One explanation, quite clear from the experimental results in this paper, is that all CGAs isomers are scavengers of the $OH^{\cdot}$ radicals. However, the mechanism of antioxidative action of CGAs isomers against $OH^{\cdot}$ radicals was rarely investigated and not fully clarified. The reaction between 5-CQA isomer and the $OH^{\cdot}$ radicals has been studied by theoretical quantum-chemical calculations (Marković & Tošović, 2016; Tošović & Marković, 2019). It was reported that the reactions of 5-CQA with $OH^{\cdot}$ radicals proceed through the four possible reaction mechanisms (pathways) depending on pH and polarity of media. The AOA mechanism of the other two CQAs isomers (4-CQA and 3-CQA) was investigated with the same conclusion as for 5-CQA isomer (Marković & Tošović, 2016). The reactions of diCQAs isomers with $OH^{\cdot}$ radicals were not investigated until now.

The second explanation could be as follows: the reaction of $OH^{\cdot}$ radicals with DNA give a wide range of different DNA radicals (such as DNA-$OH^{\cdot}$ adducts radicals, DNA base radical cations and anions, etc.) and a fast repair process takes place based on chemical reactions between the DNA radicals and antioxidants *via* an electron transfer (or H-atom transfer) processes, from antioxidants to the radical sites in a DNA damaged structure. This fast repair process can significantly diminish oxidative damage to DNA (Zheng et al., 2010). Many strong antioxidants can act in this repair process. Thus, different tea catechins protect DNA from $OH^{\cdot}$ radicals inducing strand breaks and DNA base damage through the fast chemical repair of DNA radicals (Anderson et al., 2001). The same fast repair mechanism was reported for flavonoids, rutin, and quercetin (Zhao et al., 2002), some hydroxycinnamic acids, caffeic, ferulic and sinapic acid, and many other synthetic or natural polyphenols (Zheng et al., 2010). Therefore, it can be supposed that the CGAs isomers, as very strong antioxidants, can also act *via* the fast repair mechanism in combination with the process of scavenging of $OH^{\cdot}$ radicals. However, further studies are necessary to be performed to confirm such a conclusion.

3.4. Evaluation of the antioxidant activity of aqueous coffee extracts

Electrochemical determination of the antioxidant activity of aqueous coffee extracts was performed by the same procedure and experimental conditions as those used for the CGAs isomers. Also the shapes of CVs observed after the incubation of biosensor in the cleavage mixture with coffee extracts are very similar to those observed at the CGAs isomers (not shown here). Using the normalized biosensor peak current responses $\Delta I_{a,rel}$ values obtained for *C. robusta* green bean coffee extract (as one example of the behaviour of all other coffee extracts) it can be observed that a significantly higher portion of survived DNA was found compared to that without the addition of the coffee extract. Thus, after 15 min of incubation in the mixture of cleavage agent and *C. robusta* green bean coffee extract, 72% of survived DNA was found, while in cleavage agent alone it was only 38% of survived DNA (Fig. 4).

Using the normalized biosensor responses $\Delta I_{a,rel}$, $\Delta I_{c,rel}$, and $\Delta(\Delta E_{p,rel})$ the relative portion of values of survived DNA after 15 min incubation of the DNA biosensor in a mixture of cleavage agent and some of the coffee extracts were obtained (Table 2).

From the values of relative portion of survived DNA it can be concluded that all investigated coffee extracts exhibit a significant antioxidant effect. The highest AOA was determined for the aqueous extracts of *C. robusta* and *C. arabica* green bean, while the lowest antioxidant activity was observed for the roasted coffee bean extracts. Such behaviour should correspond with the total CGAs content in the coffee extracts. Numerous HPLC analyses of different coffee samples reported a significant loss of the total CGAs content and change of CGAs profile during the roasting of green beans and further processing of ground coffees (Mills, Oruna-Concha, Mottram, Gibson, & Spencer, 2013). Recently, using several electrochemical techniques (SWV, DPV,

Table 2
Antioxidant activity of different coffee extracts towards oxidative DNA damage evaluated by the normalized CV responses $\Delta I_{a,rel}$, $\Delta I_{c,rel}$, and $\Delta(\Delta E_{p,rel})$ to express the relative portion of survived DNA at the DNA/SWCNT-COOH/SPCE biosensor. For comparison, the CGAs content determined by DPV and HPLC methods is given. Conditions: Incubation of DNA/SWCNT-COOH/SPCE in cleavage agent or a mixture of cleavage agent and some of the coffee extract for 15 min. Cleavage agent and *C. arabica* and *C. robusta* green bean coffee extracts were mixed in 1:100 (v/v) ratio, while all other coffee extracts were mixed in 1:50 (v/v) ratio. The CV record in 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ in 0.1 mol L^{-1} PB of pH 7.0 with 0.01 mol L^{-1} NaCl.

	Relative portion of survived DNA, %			CGAs content,[a,b] mg CGAs/100 g coffee	
Brand of coffee	$\Delta I_{a,rel}$	$\Delta I_{c,rel}$	$\Delta(\Delta E_p)_{rel}$	DPV	HPLC
C. robusta, Cherry, India (green bean)	72.0	71.8	68.1	9115	9112
C. arabica, Rio Minas, Brazil (green bean)	70.2	70.0	66.7	7451	7370
C. robusta, Cherry, India (roasted bean)	46.8	46.5	43.6	2826	2852
C. arabica, Rio Minas, Brazil (roasted bean)	45.7	45.6	42.5	2613	2630
Flatscher Olympia (ground coffee, 100% Arabica	53.2	53.0	51.9	4101	3932
Franck Guatemala (ground coffee, 100% Arabica)	52.7	52.6	50.2	3519	3574
Nescafé Classic (instant coffee)	49.2	49.0	46.8	3203	3283
Nescafé Espresso (instant coffee)	49.0	48.8	46.5	3185	3229
Jacobs Monarch (instant coffee)	48.6	48.6	46.2	3149	3203
Jacobs Intense (instant coffee)	49.4	49.3	47.0	3462	3465

-Results represent the mean value of three independent measurements (n = 3).
[a] Values obtained fromTomac and Šeruga (2016).
[b] Values obtained fromTomac et al. (2018).

FTCP) (Šeruga & Tomac, 2014; Tomac & Šeruga, 2016; Tomac et al., 2017) and the HPLC method (Tomac et al., 2018) we have determined the total CGAs content in the same coffee brands as investigated in the present paper. It was found that the green bean coffee extracts contain the highest total CGAs content, while the lowest CGAs content was found in the roasted bean coffee extracts. The total CGAs content reported in our previously published papers corresponds with the antioxidant activity expressed as survived DNA portion in this paper. In other words, there is a positive correlation between the AOA and total CGAs content in the investigated coffee samples.

4. Conclusions

In this work, the simply prepared DNA/SWCNT-COOH/SPCE biosensor was characterized and used for the evaluation of DNA damage induced by $OH^{\cdot}$ radicals generated by the Fenton type reaction using the cyclic voltammetry with the $[Fe(CN)_6]^{3-/4-}$ redox indicator. The proposed simple analytical procedure allowed to use this biosensor, for the first time, at the evaluation of the antioxidant activity (AOA) of six CGAs isomers and ten aqueous coffee extracts against the oxidative DNA damage. The normalized voltammetric responses of the redox indicator, i.e. $\Delta I_{a,rel}$, $\Delta I_{c,rel}$, and $\Delta(\Delta E_{p,})_{rel}$, simply evaluated after an incubation of the biosensor used in the mixture of cleavage agent and individual CGAs isomers or coffee extracts followed by the CV measurement were used to express the relative portion of survived DNA (%) as a relative criterion of AOA.

The results showed that the $OH^{\cdot}$ radicals induce strong damage to DNA structure and the extent of this damage increases with the biosensor incubation time. The addition of CGAs isomers into the cleavage agent solution significantly diminishes the degree of DNA damage. This antioxidant effect of the CGAs isomers depends on their chemical structures, especially on the presence of two *ortho*-hydroxyl (OH) groups in the catechol moiety of CGAs and the presence of ester (−COOR) group(s) in the side chain of CQAs and diCQAs. Two possible approaches can explain how the CGAs isomers reduce the oxidative DNA damage induced by $OH^{\cdot}$ radicals: (i) scavenge of the $OH^{\cdot}$ radicals by CGAs during the incubation time, (ii) direct reactions between CGAs and DNA radicals formed during processes of DNA damage that proceed through a fast chemical repair mechanism *via* the transfer of electrons (or H-atoms) from CGAs to DNA radicals (radical sites in DNA damaged structure).

All aqueous coffee extracts under investigation also exhibited the significant antioxidant activity. The highest AOA was observed for green beans coffee extracts, while the lowest antioxidant effect was observed for the roasted coffee beans extracts. Because green beans have significantly higher total CGAs content in comparison to roasted coffee beans, it seems that the AOA of coffee extracts is in some positive correlation with the total CGAs content.

The DNA/SWCNT-COOH/SPCE biosensor represents a very sensitive analytical tool which can be used very effectively for the detection of DNA damage and evaluation of the AOA of different antioxidants. Regarding its selectivity/specificity as the general important feature of the biosensors, it is fulfilled considering an advantage of the DNA strands bioaffinity properties. Generally, the nucleic acids-based biosensors are specific either to the analyte (a nucleotide bases sequence in genosensors, a protein at aptamer-based sensors) or to the nucleic acid itself (its damage) (Labuda et al., 2010). In complex sample matrices such as the coffee extracts the biosensor response reflects a group effect of chemicals (in this case the antioxidants) towards DNA. Thus, it can be concluded that the procedure and results reported here represent a contribution to further utilization of electrochemical DNA-based biosensors in analysis of food samples.

CRediT authorship contribution statement

All authors contributed equally in ideas, discussed the results and wrote the manuscript, also in conceptualization, data curation, formal analysis, investigation, methodology, writing, visualization. Authors Šeruga and Labuda contributed also in supervision, too.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by the Competence Center for SMART Technologies for Electronics and Informatics Systems and Services, ITMS 26240220072, funded by the Research & Development

Operational Programme from the ERDF.

Contributions

All authors contributed ideas, discussed the results and wrote the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.126787.

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